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Hydroacoustics for the discovery and quantification of Nassau grouper (*Epinephelus striatus*) spawning aggregations

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Abstract Fish spawning aggregations (FSAs) are vital life-history events that need to be monitored to determine the health of aggregating populations; this is especially true of the endangered Nassau grouper (*Epinephelus striatus*). Hydroacoustics were used to locate Nassau grouper FSAs at sites on the west end of Little Cayman (LCW), and east ends of Grand Cayman (GCE) and Cayman Brac (CBE). Fish abundance and biomass at each FSA were estimated via echo integration and FSA extent. Acoustic mean fish abundance estimates (±SE) on the FSA at LCW (893 ± 459) did not differ significantly from concurrent SCUBA estimates (1150 ± 75). Mean fish densities (number 1000 m^{-3}) were significantly higher at LCW (33.13 ± 5.62) than at the other sites (GCE: 7.01 ± 2.1, CBE: 4.61 ± 1.16). We investigate different acoustic post-processing options to obtain target strength (TS), and we examine the different TS to total length (TL) formulas available. The SCUBA surveys also provided measures of TL through the use of laser callipers allowing development of an in situ TS to TL formula for Nassau grouper at the LCW FSA. Application of this formula revealed mean fish TL was significantly higher at LCW (65.4 ± 0.7 cm) than GCE (60.7 ± 0.4 cm), but not CBE (61.1 ± 2.5 cm). Use of the empirical TS to TL formula resulted in underestimation of fish length in comparison with diver measurements, highlighting the benefits of secondary length data and deriving specific TS to TL formulas for each population. FSA location examined with reference to seasonal marine protected areas (Designated Grouper Spawning Areas) showed FSAs were partially outside these areas at GCE and very close to the boundary at CBE. As FSAs often occur at the limits of safe diving operations, hydroacoustic technology provides an alternative method to monitor and inform future management of aggregating fish species.

Keywords Hydroacoustics · Nassau grouper (*Epinephelus striatus*) · Fish spawning aggregations (FSAs) · Echo integration

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Introduction

Fish spawning aggregations (FSAs) are broadly defined as 'a group of conspecific fish gathered for the purposes of spawning with fish densities significantly higher than are found during the non-reproductive periods' (Domeier and Colin 1997). This reproductive strategy creates temporary concentrations of fish (Johannes 1978; Kobara and Heyman 2008) that are highly susceptible to overfishing (Nemeth 2005; Starr et al. 2007; Sadovy de Mitcheson and Erisman 2012). The health of a FSA is a good indicator of the health of the population as a whole (Gascoigne 2002), and any depletion of a FSA has serious consequences for the reproductive output of that population (Sadovy and Domeier 2005; Sadovy de Mitcheson 2016). FSAs therefore are important life-history phenomena that must be considered in any efforts to manage fisheries of aggregating species (Sadovy and Colin 2012; Sadovy de Mitcheson 2016). We use the term FSA for fish that are gathered together for the purpose of spawning. We acknowledge, however, that the aggregations of fish detected may not have been spawning per se at the specific times of the surveys.

One of the best known examples of the demise of a species due to FSA over fishing is that of the Nassau grouper (*Epinephelus striatus*) (Sadovy de Mitcheson et al. 2008). These large top-level predators are an important species within Caribbean reef ecosystems (Stallings 2008, 2009; Archer et al. 2012). Nassau grouper migrate to specific sites during periods of winter full moons to reproduce in FSAs (Sala et al. 2001; Whaylen et al. 2004; Starr et al. 2007) and were one of the first large-bodied tropical reef-fish species scientifically documented to do so (Smith 1972). It is estimated that 75% of all known Nassau grouper spawning aggregations have either been eradicated or reduced to negligible numbers (Sadovy de Mitcheson et al. 2008). Following over-exploitation, these aggregations often fail to recover (Gibson 2007; Semmens et al. 2007), although recent evidence suggests that effective management can lead to population increases (Kadison et al. 2010; Heppell et al. 2012). FSAs in the Cayman Islands have been reported on the eastern and southwest points of Grand Cayman, the northeast and southwest points of Little Cayman and the southwest point of Cayman Brac (Bush et al. 2006). These sites were protected by legislation in 2003 which prohibits fishing in these areas (Whaylen et al. 2006), and due to winter spawning, it is now forbidden to take a Nassau grouper from Cayman waters during the months of December to April (Cayman Islands Government 2016).

Monitoring spawning aggregations

AQ1 Monitoring an FSA is an effective way to determine the health of an aggregating population, but adequately monitoring an FSA requires a clear understanding of its location, extent, and dynamics. In-water monitoring is fraught with difficulties including high temporal variability in fish numbers and variable distribution across multiple sites, the expense of underwater visual census (UVC) surveys and challenging underwater working conditions (including strong currents, poor visibility and FSA locations below safe diver depth limits) (Sadovy and Domeier 2005). This is especially true in the Cayman Islands where FSAs occur on the extreme tips of the islands at locations where currents are strong and dives must occur at dawn and dusk to coincide with periods of peak fish activity. Further, observer bias may be present in UVC surveys and fish may avoid divers (Colin 1992; Murphy and Jenkins 2010).

Hydroacoustics may be useful for assessing aggregating reef fishes that are otherwise difficult to count (Johannes et al. 1999). One of the main advantages of hydroacoustics is the ability to collect large volumes of information in a short amount of time (Trenkel et al. 2011; Jones et al. 2012). Further, unlike video or UVC, the acoustic technique is unaffected by underwater visibility (Gledhill et al. 1996) nor are the fish influenced by the presence of a diver. To date there has been limited use of hydroacoustics to monitor spawning aggregations (e.g. Johnston et al. 2006; Taylor et al. 2006; Ehrhardt and Deleveaux 2007) and Taylor et al. (2006) noted the technology can provide an accurate estimate of overall fish abundance and spatial extent in comparison with diver visual counts. Studies comparing hydroacoustics and UVC are sparse, however. Taylor et al. (2006) reported similar acoustic density and diver estimates over their entire survey region, although total abundances differed likely due to differences in area covered by the two methods and the patchy distribution of the fish. Although hydroacoustic techniques hold great promise, many authors highlight that ground-truthing is required to identify the fish to species level (Simmonds and MacLennan 2005; Ryan et al. 2009).

The International Union for the Conservation of Nature (IUCN) lists the Nassau grouper as endangered and recommends annual monitoring at as many traditional aggregation sites as possible, including adjacent areas where aggregations have not previously been reported and as part of the assessment of the effectiveness of protected areas (Carpenter et al. 2015). Given the need to develop effective monitoring techniques that can rapidly, effectively, and quantitatively assess FSA status, we investigated the capacity of hydroacoustics to address these recommendations. We examined FSA locations in relation to protected zones in the Cayman Islands and compared acoustic data with diver-collected data. Further, we evaluated the different acoustic processing methods available to estimate the sizes of fish within FSAs.

Materials and methods

Survey sites

The sites chosen in this study are all within the Designated Grouper Spawning Areas (DGSA) of the Cayman Islands. Surveys were focussed on the likely areas of the FSA, based on site geomorphology and from local knowledge via the Department of Environment (DoE) (Fig. 1). Most survey effort was concentrated on the FSA located at the

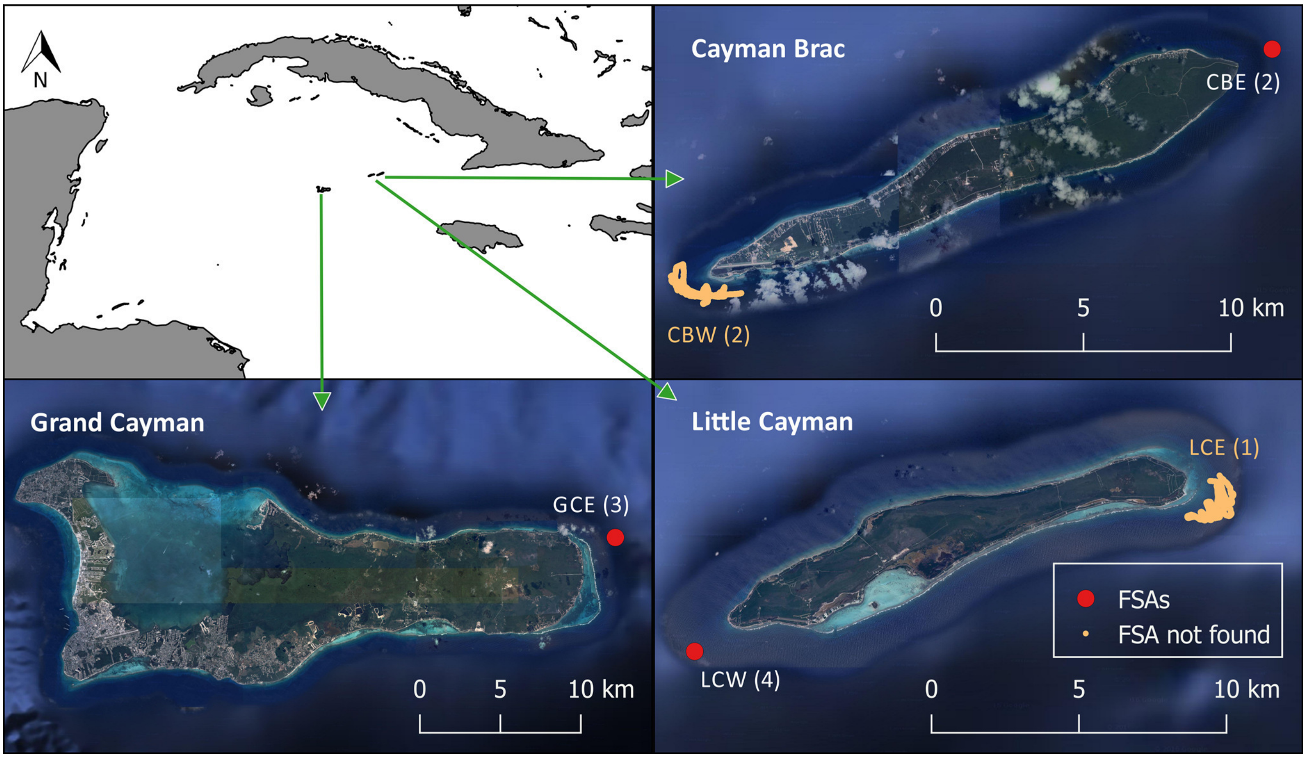

Fig. 1 Areas in the Cayman Islands surveyed by hydroacoustics and in-water assessment techniques The numbers at each site represent the total number of hydroacoustic surveys undertaken at each location. *Red dots* show located fish spawning aggregations (FSAs); *peach colour* shows survey tracks that did not locate FSAs. Map data ©2016 Google

Table 1 Dates and times of the surveys conducted, with the number of days elapsed since the February full moon

Survey name	Date	Start time	Stop time	Days after full moon
GCE1	14/02/2014	12:40:43	15:19:39	0
LCE1	15/02/2014	17:48:01	19:33:54	1
LCW1	16/02/2014	12:04:38	12:52:39	2
LCW2	16/02/2014	17:38:42	17:51:19	2
LCW3	16/02/2014	18:38:18	19:12:52	2
LCW4	17/02/2014	13:24:40	13:55:05	3
CBW	17/02/2014	17:05:56	18:25:45	3
CBE	18/02/2014	17:44:52	19:00:25	4
CBW2	18/02/2014	10:43:05	13:04:03	4
CBE2	19/02/2014	07:43:09	08:48:04	5
GCE2	19/02/2014	17:13:11	18:28:32	5
GCE3	20/02/2014	08:13:58	09:41:08	6

Times are in Easter Standard Time (EST) (UTC/GMT -5 h)

west end of Little Cayman (LCW) as this is known to be the most active of the FSAs, and for which concurrent fish abundance and size data obtained via SCUBA were provided by the Grouper Moon project (http://www.reef.org/groupermoonproject). Surveys were also conducted at Little Cayman East (LCE), Grand Cayman East (GCE) and Cayman Brac West (CBW) and East (CBE). The field surveys in Cayman occurred between 14 and 20 February 2014 (Table 1).

Equipment

A Biosonics DTX split-beam echosounder with a 200-kHz transducer (beam opening angle of 6.8°), pole mounted over the side of the survey vessel, was used for the surveys. Data were collected with Biosonics visual acquisition software (Biosonics Inc., Seattle, WA). Pulse duration was 0.4 ms, and the specified ping rate was 10 s^{-1}. Survey speed was kept to approximately 4 kn and sea state was calm (Beaufort scale 3 or under) on all surveys. The echosounder was calibrated before the start of the surveys on 13 February 2014 using a tungsten carbide 36-mm standard calibration sphere, following the standard methods (Foote et al. 1987; Demer et al. 2015). The acoustic return from the sphere was within acceptable tolerance to the expected value given for the local environmental settings (TS = −39.6 vs. −39.8 dB, respectively (Biosonics 2004), with speed of sound calculated as 1521.54 m s^{-1}). Where diver observations were not available for species ground-truthing, underwater video was used (Thomas and Thorne 2003; Doray et al. 2007; Jones et al. 2012). This consisted of a Sony 37CSHR camera with a live surface feed mounted on an aluminium wing. Both the acoustic data and the video data were time-stamped allowing syncing of the visual and acoustic records in post-processing.

Data processing

Potential Nassau grouper FSAs were initially identified through their stronger backscattering properties and school morphology (Fig. 2) than aggregations of other species (e.g. horse-eye jack, *Caranx latus*) and then verified by visual observation either by the use of the pelagic tow camera or through confirmation by the dive team at LCW.

Data were processed with the software package Sonar5-Pro (Balk and Lindem 2006), following the software-guided analysis routine (see Parker-Stetter et al. 2009 for details). The analysis was based upon echo integration (also known as Sv/TS scaling) which divides the average reflection from all fish over a segment (the volume backscattering coefficient, Sv) by the average target strength (TS) from individual fish (Winfield et al. 2011). TS is defined as TS $= m\mathrm{Log}L + b$ where m and b are constants for a given species and frequency, respectively, and $L =$ length as total length (TL), (Simmonds and MacLennan 2005). Initially, a threshold of −60 dB was applied to the echograms to distinguish fishes from other particulate targets such as plankton. This is a typical threshold applied for the detection of pelagic schooling fishes (Reid 2000). Any noise due to issues such as bubbles in the water column from wave action was removed by eye. Sonar5 applies a time-varied gain correction of 40log(R) for TS values and 20log(R) for Sv values (Balk and Lindem 2006). A bottom exclusion layer of 1 m was applied, and data from within this layer were not included in the analysis due to the 'acoustic dead zone' (Ona and Mitson 1996). For echo integration methodology, there are two main options to obtain TS: using tracked fish as a source or using 'single echoes detected' (SED) as source. We used tracked fish as source to derive abundance estimates but examined the efficacy of both options to derive TS. We used the following criteria to track fish within the FSAs: a minimum track length of three pings; a maximum ping gap of two pings: a gating range of 0.3 m; a maximum mean echo threshold of −25 dB; and a minimum mean echo threshold of −40 dB. Due to difficulties in obtaining sufficient numbers of tracks from within FSAs (likely due to high fish density and low signal-to-noise ratios in dense areas of the aggregation), tracks were extracted and stored from all passes of the FSAs per survey and then the tracked fish were used to provide the survey-specific abundance estimates. As tilt angle of fish can have a significant bearing on TS, extreme tilt angles were filtered out of the data following Gauthier and Horne (2004), so that any fish with an aspect ±40° from horizontal (dorsal aspect) were removed from the analysis. We examined both the mean TS of fish echoes in each track (calculated in the linear domain) and the 75th percentile of TSs of each track. For fish TS estimates using SED as source, SED were extracted for each pass of an FSA and mean TS values subsequently determined for the FSA from each survey. To assess whether fish near the top of a school were shadowing those beneath them, data were checked to ensure that echo energy was consistent from the top to the bottom of the school following Knudsen et al. (2009) (see electronic supplementary information, ESM, Fig. S1).

Three main equations were examined to convert TS to fish TL by applying our mean TSs values (Table 2). Further, we scaled diver fish length (TL) measurements (taken using a laser calliper system; Heppell et al. 2012) by our mean TS data from tracked fish for the LCW FSA, by sorting both datasets by increasing value and then plotting one against the other to determine a survey-specific TS–TL formula (see ESM Fig. S2) resulting in Formula 4 in Table 2.

TL—weight regressions specific to the Nassau grouper—were used to calculate weight at TL for biomass estimates using the formula $W = aLb$ where $W =$ weight (g), $L =$ TL (cm), $a = 0.01122$, $b = 3.05$ (Froese and Pauly 2016).

Applying the TS–TL formula and then using the specific TL-to-weight relationship for the Nassau grouper (Froese and Pauly 2016) give the mean weight of fish in each FSA. This number was then multiplied by the number of fish estimated in each FSA to provide total biomass estimates for each FSA surveyed.

Spatial extents

Once the FSA was located using preliminary acoustic transects, the aggregation was surveyed from different angles to corroborate its extent. This approach follows Doonan et al. (2003), who noted the advantages of a star-shaped survey track in hydroacoustic surveys over

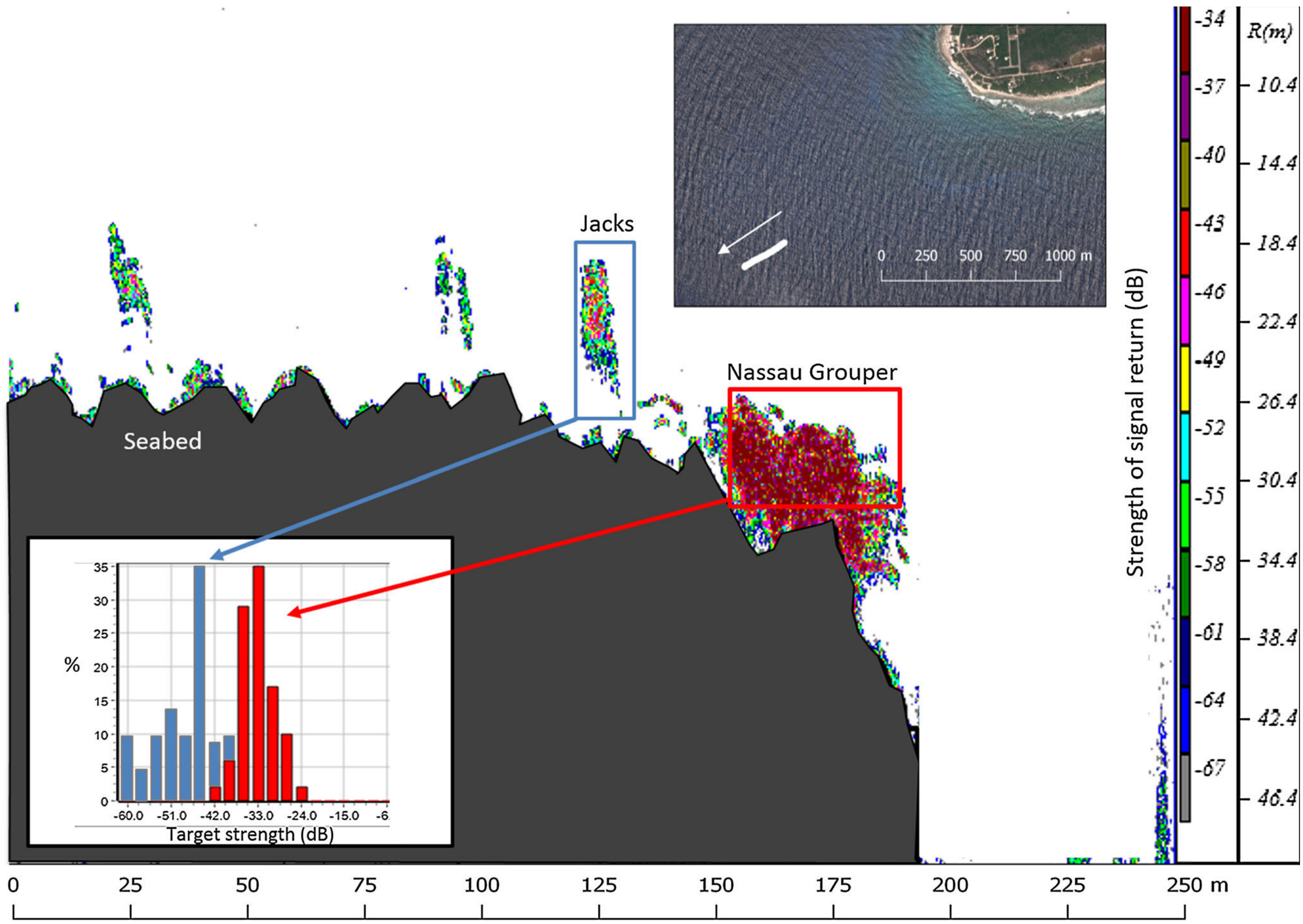

Fig. 2 An example echogram of the analysis of fish echoes resulting from a Nassau grouper fish spawning aggregation (FSA) (*red*) and those from an aggregation of horse-eye jacks (*blue*). The *inset* shows that grouper had a higher percentage of stronger echoes. Transect distance is shown along the *x-axis*, while depth [R(m)] and strength of signal return (*colour strip*) are shown on the *y-axis*. The satellite image shows the location of the transect of the Little Cayman west (LCW) 1 survey, and the *arrow* shows the direction of travel. Map data ©2016 Google

Table 2 Target strength (TS) to length (L) formulae examined in this study

	Formula TS to L	Formula L to TS	Reference	Species	Frequency (kHz)
1	TS = 19.1 log10(L) − 64.07	L = (2261.8)*EXP[0.1206*(TS)]	Love (1971)	Multi species	200
2	TS = 0.7091*L − 89.136	L = (TS/0.7091) + 89.136	Ehrhardt and Deleveaux (2007)	*Epinephelus striatus*	200
3a	TS = 19.2 log10(L) − 64.05	L = (2165)*EXP[0.12*(TS)]	Rivera et al. (2010)	*Epinephelus guttatus*	120
3b[a]	TS = 19.2 log10(L)−64.25	L = (2220)*EXP[0.1199*(TS)]	Rivera et al. (2010)	*Epinephelus guttatus*	200
4	TS = 27.6 log10(L) − 147.32	L = (207.06)*EXP[0.0362*(TS)]	This study	*Epinephelus striatus*	200

Length is total length in cm

[a] 3b is 3a reformulated for 200 kHz

schooling fishes. Alongside fish abundance values, the geographical extents were also extracted, but these are given in only two dimensions (height and length). Where survey tracks crossed the FSA from different angles, the full three-dimensional extent of the FSA was estimated by drawing a polygon (Fig. 3) as per the arithmetic extrapolation method used by Taylor et al. (2006) and Ehrhardt and Deleveaux (2007). When the track crossed the FSA from only one angle, it was assumed that the aggregation was circular unless nearby pings showed no fish were present, in which case the halfway point between the positive (FSA detected) and negative (FSA not detected) pings was taken to demarcate the FSA extent. If the FSA represented two or more clear densities, separate polygons were drawn for each density class present. Once a polygon was drawn, fish abundance was calculated by multiplying the mean number of fish ha^{-1} by the area of the polygon. When there were multiple polygons of differing abundances, the result of each was summed to give a total number of fish.

Statistical analyses

Welch's ANOVAs (equal variances were not assumed) were used to compare fish densities (number of fish 1000 m^{-3}, log transformed) among sites and surveys at LCW, and a two-sample *t* test was used to compare densities at GCE surveys. Diver fish abundance estimates were compared to the acoustic abundance estimates by using a two-sample *t* test. The TS values from the different acoustic processing methods were compared for each site with two-sample *t* tests. Values of fish TL gained from applying tracked fish mean TS data coupled with our in situ formula were compared among the different surveys and sites with Welch's ANOVA, and Games–Howell pairwise comparisons were used to test where the differences among sites existed.

Results

Numbers of fish in each FSA

FSAs were identified at LCW (all four surveys), GCE (two of three surveys) and CBE (one of two surveys). No FSAs were detected in the surveys of CBW or LCE. Visual confirmation that the targets were Nassau grouper was provided by the Grouper Moon dive team at LCW and at GCE by the towed camera system. We did not achieve visual confirmation of species present at CBE; however, mean TS's and FSA morphology at that location were similar to those at the verified Nassau grouper FSA sites. The highest acoustically measured fish abundance was detected at LCW with a maximum abundance of 2194 fish in the aggregation (survey LCW1) 2 d after the full moon on 16 February 2014. Fish density was significantly greater at LCW FSA than at the other two sites ($F_2 = 25.49$, $p = 0.000$) which did not differ significantly from each other. Fish densities did not differ significantly among individual surveys at the LCW FSA ($F_3 = 1.35$, $p = 0.319$) or the GCE FSA ($T_8 = 1$, $p = 0.349$) (Table 3).

Comparison between acoustic and diver abundance data

Diver-estimated numbers of fish at the LCW FSA were made concurrent with acoustic surveys LCW2, LCW3 and LCW4 (Table 3). Diver confirmation of species also occurred during LCW1, although numbers could not be recorded. No significant difference was detected at the 95% confidence level between diver estimates and acoustics ($T_3 = 0.55$, $p = 0.619$).

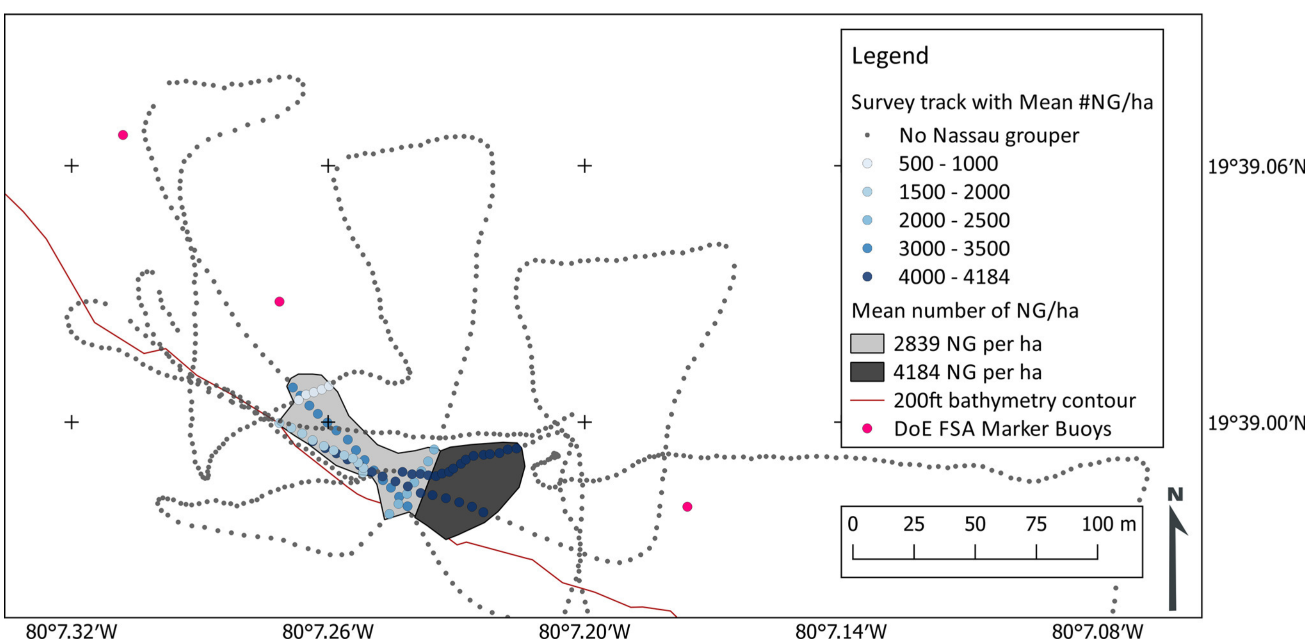

Fig. 3 Example of fish spawning aggregation (FSA) polygon determination in the arithmetic extrapolation method during the Little Cayman west (LCW) 4 survey. *NG* Nassau grouper. Department of Environment Little Cayman FSA location marker buoys shown in *pink* and the 200 ft bathymetry contour shown in *brown*. *Crosses* indicate where latitude and longitude intersect

Fish TS

Mean fish TS gained through tracked fish was compared with mean fish TS via SED for each site (Fig. 4). There was no significant difference in mean TS values at any site (CBE: $T_{12} = 0.03$, $p = 0.98$, LCW: $T_{47} = 1.44$, $p = 0.157$, GCE: $T_{28} = 0.59$, $p = 0.557$). The TS values from the 75th percentile of echoes in a fish track were significantly higher than the mean TS at LCW ($T_{192} = 3.78$, $p = 0.000$) and GCE ($T_{429} = 6.91$, $p = 0.000$), but not at CBE ($T_{19} = 1.13$, $p = 0.273$) presumably due to the smaller number of observations reducing statistical power.

Converting TS to TL

Mean TS measurements from tracked fish were scaled by the diver LCW FSA diver length data. This resulted in: TS $= 27.6\log10(L) - 147.32$ ($R^2 = 0.98$; ESM Fig. S2). The results from applying this formula to TS data are plotted for the LCW dataset alongside the alternative equations given in Table 2 (Fig. 5).

The results of applying our in situ formula to the acoustic TS data are plotted per individual survey (Fig. 6a) and as mean values per site (Fig. 6b).

There was a significant difference in mean fish TL calculated from mean TS of tracked fish between the sites ($F_2 = 15.08$, $p = 0.000$), with significantly larger fish at LCW than at GCE but not CBE, which did not differ from each other. Using the von Bertalanffy growth curve for the Nassau grouper sampled from aggregations in the Cayman Islands 1987–1992 (Bush et al. 2006), the estimated mean fish TL of 65.4 ± 0.7 cm seen at the LCW FSA corresponds to an age of 10 yr. The estimated mean sizes of fish at the GCE FSA (60.7 ± 0.4 cm) and CBE (61.1 ± 2.5 cm) correspond to those of 8-year-old fish.

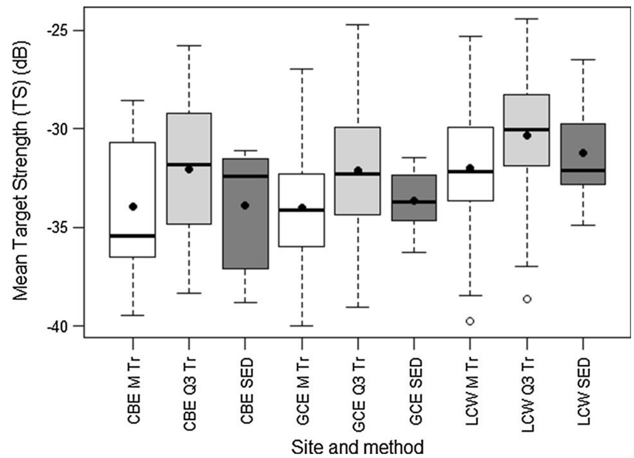

Fig. 4 Mean fish target strength (TS) found in fish spawning aggregations during each survey, per site and for each of the acoustic processing methods. *CBE* Grand Cayman Brac, *GCE* Grand Cayman east, *LCW* Little Cayman west, *Tr M* mean echo of tracked fish, *Q3 Tr* 75th percentile of echoes from tracked fish, *SED* single echoes detected. *Box plots* show mean values (*black circle*), median values (*solid horizontal line*), and the *lower and upper* ends of the box are the 25 and 75% quartiles, respectively. The whiskers indicate 1.5 times the inter-quartile range, and points beyond this range are shown by *empty circles*

FSA location relative to Cayman Islands DoE Designated Grouper Spawning Areas

The extent of the FSA located on Grand Cayman fell on the extreme northern limit of the DGSA boundary on the

Table 3 Estimates of mean TS, mean lengths, weights, fish numbers and subsequent biomass values per survey where a FSA was identified as derived from mean TS from tracked fish

Survey name	Mean TS (dB)	Mean length (cm)	Mean weight (g)	Fish number	Biomass (kg)	Verification method	Fish density (#/ 1000 m^3)	Fish number/ isonified volume (Nv)	Mean depth (m)
LCW1	−31.98 (0.86)	65.22 (2.06)	3900.03 (390.9)	2194	8556.67	D (NP)	46.89 (24.60)	0.095 (0.05)	28.0 (1.4)
LCW2	−32.89 (1.43)	63.60 (3.30)	3782.35 (598.3)	398	1505.37	D (1225)	24.69 (12.76)	0.051 (0.024)	28.9 (2.1)
LCW3	−32.62 (1.25)	63.94 (2.97)	3746.54 (559.0)	122	457.08	D (1225)	18.20 (5.29)	0.031 (0.007)	26.2 (2.6)
LCW4	−30.50 (0.84)	68.86 (2.11)	4615.64 (443.8)	857	3955.60	D (1000)	32.87 (21.50)	0.072 (0.046)	29.0 (2.6)
LCW all	−32.01 (0.61)	65.40 (1.44)	4018.20 (268.1)	893	3588.25	D	33.13 (11.02)	0.067 (0.023)	28.1 (1.1)
CBE1	−33.95 (2.26)	61.12 (5.08)	3327.10 (849.2)	58	192.97	NP	4.61 (2.27)	0.009 (0.005)	30.4 (1.9)
GCE2	−33.95 (0.55)	60.90 (1.2)	3208.22 (191.6)	49	157.20	TC	4.01 (2.24)	0.0198 (0.011)	43.7 (2.2)
GCE3	−34.07 (0.48)	60.61 (1.08)	3162.43 (181.7)	40	126.50	TC	8.37 (5.82)	0.042 (0.028)	46.1 (1.1)
GCE all	−34.01 (0.36)	60.74 (0.8)	3183.32 (131.6)	45	143.25	TC	7.01 (4.12)	0.035 (0.019)	45.2 (1.1)

Fish density is number of fish per 1000 m^3. Nv is number of fish per volume isonified (Sawada et al. 1993). Verification method shows how the fish were identified *D* diver (number in brackets), *NP* not possible, *TC* towed camera. Mean depth is the mean fish depth at each FSA. Numbers in brackets are 95% confidence levels

GCE2 survey and just outside the boundary during the GCE3 survey. At CBE, the FSA was just within the boundary close to its northern limit. The LCW FSA was within the associated protection zone (Fig. 7).

Discussion

The greatest fish abundances and densities were recorded at the LCW FSA. This is as expected as this particular FSA is well known throughout the Caribbean for the high numbers of fish present there during spawning periods (Whaylen et al. 2004). It should be noted that these surveys occurred closest to the full moon (2–3 d after the full moon), when Nassau grouper FSAs are most active (Starr et al. 2007). The surveys LCW1 and LCW4 both yielded very similar patterns of fish distribution and had the highest abundance estimates. These surveys occurred at similar times near the middle of the day, while surveys LCW2 and LCW3, both occurring near dusk, recorded lower abundances. Other studies have found that groupers were more densely aggregated at sunrise and sunset (Whaylen et al. 2006), and it is possible that the main aggregation may therefore have been missed by surveys LCW2 and LCW3, or that abundance estimates are more robust when fish are more dispersed as has been seen in other studies (Rudstam et al. 2003).

At any given time in the LCW FSA, some proportion of the fish are located on the plateau and across a wider area than is represented by the main aggregation at the reef crest (Whaylen et al. 2006); it is possible that the acoustics may not have detected these individuals. In addition, as fish within 1 m of the seabed were not included in the study, acoustic abundance estimates are best considered an index of abundance rather than an absolute abundance and are likely to be conservative compared to the total number of all spawning fish. The LCW FSA was most active the day before the acoustic surveys (15 February, 1 d after the full moon) with 4000 fish estimated by the dive team. Our peak number of fish was detected the following day. The CBE FSA was surveyed 4 d after the full moon, and the FSA at GCE surveyed 5 and 6 d after the full moon; only small numbers of fish were found at either location. It is likely that the acoustics results underestimate the total abundances of individuals in these FSAs as they do not account for the most active times, i.e. closer to the full moon. Therefore, we recommend that to fully evaluate a given FSA, acoustic surveys should be conducted both over several days and at multiple times per day to increase the probability of capturing peak abundance at any given FSA. Note that we assumed that all echoes from within a FSA were Nassau grouper, but it is possible that relatively low numbers of other fish species were also present.

We evaluated the possibility of acoustic shadowing leading to the differences between diver estimates and acoustic estimates of fish numbers. No decrease in echo energy from the top of the FSAs to the bottom was found, indicating that the acoustic technique can be used to accurately quantify fish in FSAs (Knudsen et al. 2009). However, this is contrary to some other studies which have reported a shadowing effect in dense schools of marine fishes (Zhao and Ona 2003; Utne and Ona 2006; Løland et al. 2007).

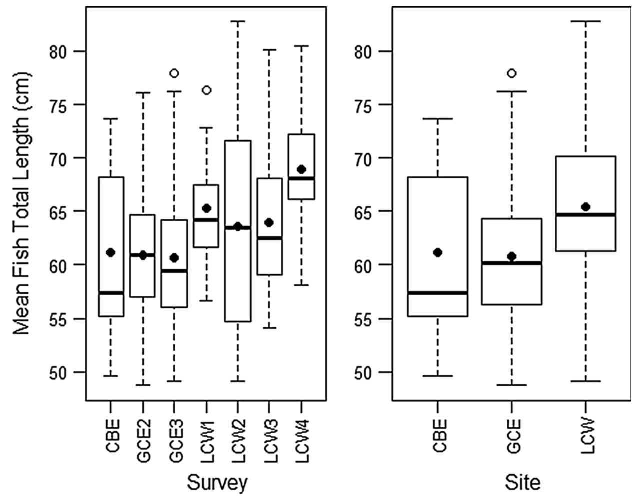

Fig. 6 Mean fish total length (TL) as calculated by applying our in situ formula **a** during each survey and **b** as grouped data per site. *Box plots* show median values (*solid horizontal line*), and the *lower and upper* ends of the box are the 25and 75% quartiles, respectively. The whiskers indicate 1.5 times the inter-quartile range and points beyond this range are shown by *empty circles*

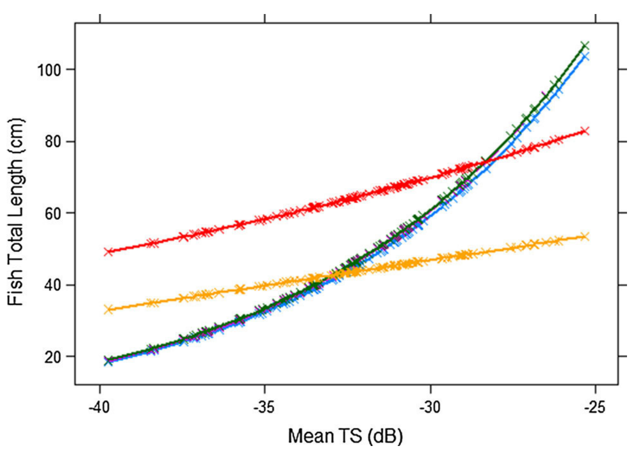

Fig. 5 Target Strength (TS) data from the Little Cayman west (LCW) surveys and corresponding fish total length using the following empirical formulas: TS = 19.2log10(L) − 64.05(*blue*; Rivera et al. 2010): TS = 19.1log10(L) − 64.07 (*pink*, partially hidden due to similar values as *green*; Love 1971),: TS = 0.7091 ∗ L − 89.136 (*yellow*; Erhardt and Deleveaux 2007), TS = 27.6log10(L) − 147.32 (*red*, this study)

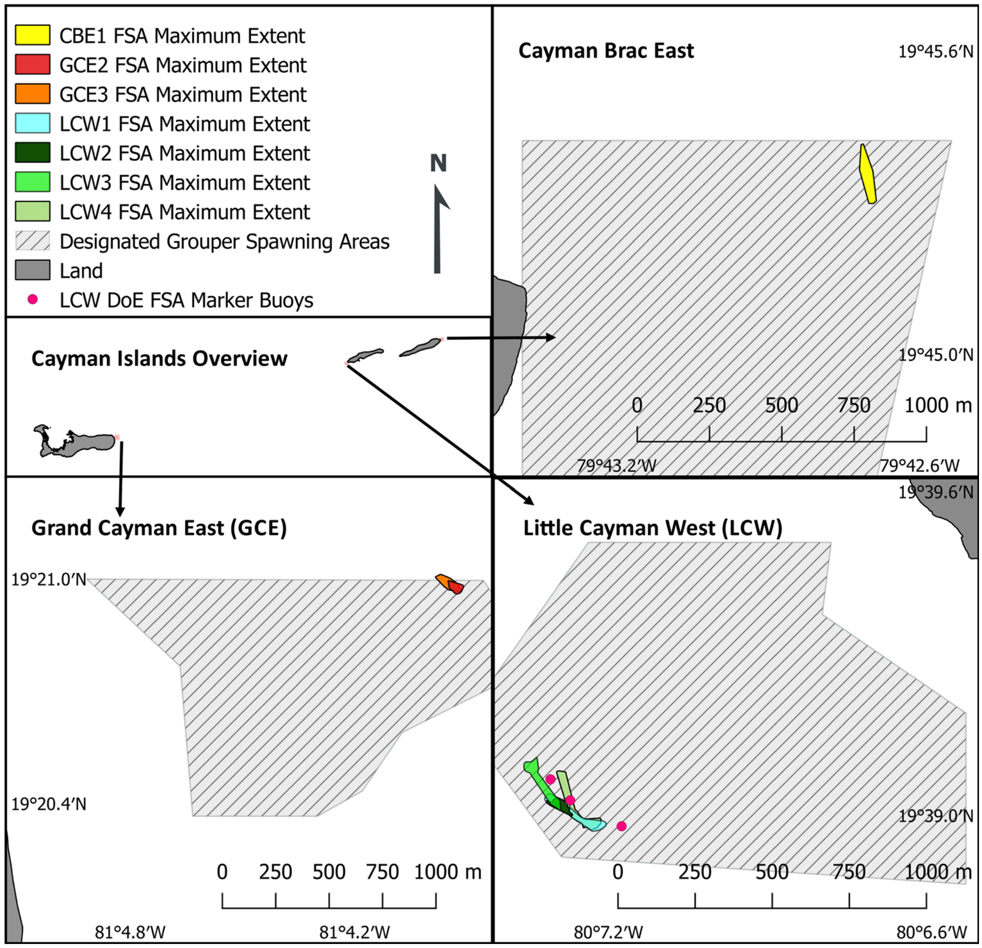

Fig. 7 Fish spawning aggregation locations and maximum extents detected via hydroacoustics in the Cayman Islands in relation to the positions of the Designated Grouper Spawning Areas (*hatched area*)

We examined three different methods in the acoustic post-processing to extract TS values, and it is interesting to note that mean TS with SED as source did not differ significantly from the mean TS of tracked fish. When fish are tilted further from the horizontal, TS is reduced so max TS may be a better estimator than mean TS (Balk and Lindem 2006). However, to remove any effect of 'flash echoes' (Lilja et al. 2004) and also the potential exaggerating effects on mean TS of multiple echoes (Soule et al. 1995; Rudstam et al. 2003), a 75th percentile of the TS along a tracked fish was also examined and unsurprisingly yielded higher values overall than the other two methods. However, we used the mean TS for subsequent calculations as this method is most common in the literature (e.g. Guillard et al. 2004; Rose 2009).

TS varies with tilt angle (Nielsen and Lundgren 1999), and among fish species due to anatomical differences in the size of the swim bladder (Simmonds and MacLennan 2005). Therefore, an empirical TS–TL relationship is needed to convert TS to fish TL, which is known for many species (Kracker 2007). Ideally, TS data should be obtained from fish that are typical of the population to be surveyed (Simmonds and MacLennan 2005). The LCW FSA presented a rare opportunity to do this as the fish species (almost entirely Nassau grouper) could be determined by divers who were also able to provide accurate length measurements. By scaling our TS values by the diver measurements, we derived an alternative in situ TS–TL equation allowing comparison to the other equations examined. Application of either the Love (1971) or Rivera et al. (2010) formula results in a significant underestimation of fish size in comparison with the diver data. Although our equation contains a log function, it is more similar to the Erhardt and Deleveaux (Ehrhardt and Deleveaux 2007) than the other equations. This is likely to be due to the relatively narrow range of fish sizes in both their and our studies, as these are the lengths of reproductively active fish. While applying our equation matches diver lengths at LCW, we are hesitant to suggest without further evaluation that it should be used in preference to other equations in future studies due to a number of reasons. First, there was a relatively narrow range of fish lengths present in the FSA as seen by divers, and applying our formula may have the effect of overestimating the size of smaller fish and underestimating the size of larger fish beyond the range experienced here. Second, there are difficulties in extracting tracked fish TS data from the centre of FSAs and it may be the case that the tracked fish, more commonly located on the periphery of the aggregation, may be of a different size or orientation than those in the centre (Starr et al. 1995). Third, tracking fish is difficult in vertical marine applications (Guillard et al. 2004), and although we experienced calm sea states, vessel movement is likely to have reduced the number of possible tracks and increased variation in TS. We recommend further examination of the TS–TL relationship for Nassau grouper and that caged fish experiments, or similar, should be conducted across a larger range of fish sizes to obtain more empirical data points from which a potentially more robust equation can be determined. Future research examining the novel combination of hydroacoustics and laser callipers could prove useful for FSA monitoring and other assessments of fish populations. The effect of reproductive state on TS of Nassau grouper would also be worthy of examination, since the relationship of gonad size to swim bladder volume of spawning sardines is as important as the relationship of the swim bladder volume to fish length (Machias and Tsimenidis 1995). Mean fish TL was significantly larger at LCW than at GCE, but not CBE. As younger fish tend to be smaller, a recovering population may have a larger proportion of smaller fish (Heppell et al. 2012). Our results could indicate that the FSAs on GCE and CBE may be recovering from previous exploitation (Bush et al. 2006) or that the generally smaller fish at those locations are a result of larger fish being removed by fishing.

Hydroacoustics allowed us to determine the location of FSAs in three-dimensional space. Spawning aggregations were consistently found just off the reef crest at around 30 m depth at LCW as has been described previously by direct observation (Whaylen et al. 2004). The depths of FSAs will be influenced by a number of factors such as diurnal time of survey or lunar phase (Starr et al. 2007); however, knowing the depths from our surveys may assist managers in determining optimum future survey strategies. The relatively deep FSA of GCE was also noted by Kobara and Heyman (2008) and is most likely due to the spawning suitability of the local geomorphologic characteristics at the site. The depth at which this FSA occurs highlights the difficulty of visual census approaches using SCUBA. FSAs can move between repeat surveys within the same lunar period, and some wider movement not detected in this study could reasonably be expected. We recommend including line fishing in the one-mile-radius restrictive buffers around DGSAs or increasing the size of the DGSAs as a further precautionary measure. If fishing occurs at the edge of the protected areas, as is common practice following closures to fishing (Kellner et al. 2007), it is possible that these FSAs, which may be recovering, could still be at risk.

Hydroacoustics has proven capable of locating FSAs in historic areas where it was unknown whether fish were still aggregating. This also means that acoustics can be used to search for aggregations in new locations and used in situations when diving surveys are impractical or hazardous. We have shown that surveying FSAs with hydroacoustics produces fish count information comparable to that from diver estimates, and it provides additional information such as fish size when ground-truthing is also provided, although further work is needed in this area. Repeating hydroacoustics surveys could yield much information on how exploited FSAs are recovering and could assist with the vital monitoring of endangered aggregating populations.

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