**Electronic Supplementary Material For:**

**A sound worth saving: acoustic characteristics of a massive fish spawning aggregation**

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**Supplementary Methods**

All data collection and methods were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California San Diego under IACUC protocol S13240.

*Survey Design*

In concurrence with the known peak spawning period for Gulf corvina (*Cynoscion othonopterus*), e.g. 1 – 3 days before the new and full moons on outgoing tides [1], we conducted four synchronous surveys of fish densities and sound production across the spawning grounds in the northeastern channel of the Colorado River Delta, México using active and passive acoustics from two separate fishing vessels (8 m long). Surveys were conducted during outgoing tides on the afternoons of 27 and 28 March 2014 and 27 and 28 April 2014 (1 – 3 days before the new moons). During each survey, the first vessel, outfitted with an active acoustic echosounder, conducted semi-randomized parallel transects across the delta channel approximately every 0.5 km to estimate the spatial distribution and abundance of corvina. The second vessel, equipped with passive acoustic instrumentation, recorded ambient sound at a random location along each active acoustic transect to measure, characterize, and map sound production of corvina. Additional passive acoustic measurements were made outside of the spawning period (November) at four locations across the study site to estimate ambient sound pressure levels in the absence of corvina during outgoing tides.

*Active Acoustic Instrumentation, Sampling, and Analysis*

A 120-kHz echosounder (ES60, Simrad-Kongsberg, Norway) configured with a 9° split-beam transducer (ES120-F, Simrad-Kongsberg, Norway) was used to conduct active acoustic surveys. The complete system was calibrated using the standard sphere method [2, 3] and a 38.1 mm diameter sphere made of tungsten carbide with 6% cobalt binder material (Bal-tecTM, Micro Surface Engineering, Inc., USA). During each survey, we sampled water temperature and salinity profiles using a CTD (Castaway®-CTD, SonTek/Xylem, Inc., USA). Profiles were used to calculate sound speeds and absorption coefficients, enabling the calibration of data during processing. During surveys, the echosounder functioned with a pulse duration of 256 µs, a ping rate of 0.25 s per transmission, and a transmit power of 200 W. The transducer was located 0.53 m below the sea surface with the beam axis oriented 10° below horizontal to permit an increased insonified volume and range while negating an interaction with the sea surface. Vessel speed was approximately 6 knots during data collection, and received power and split-beam phase data were sampled every 64 µs and stored with time and geographic location.

Active acoustic data were calibrated and analyzed in a commercial software (Echoview V5.4, Echoview Software Pty Ltd, Australia). Seabed echoes, near-field range (0.51 m), and regions of noise were removed from analyses using automatic detection algorithms and manual editing. We excluded portions of data collected in transit between transects, resulting in a series of parallel transects. We used a single detection operator (Split Beam Method 2, Echoview Software Pty Ltd, Australia) and target strength (TS) and angular-position operands to identify single targets resulting from the insonification of corvina. A minimum TS of -46.5 dB was utilized in the detection operator based on a knowledge of the minimum sizes of mature corvina expected to be present [4, 5] and the modelled, side-aspect TS versus total length (TL) relationship for the species [6]. Individual corvina were identified as tracks of two or more single targets using a tracking algorithm (Alpha-Beta, Echoview Software Pty Ltd, Australia) configured with limits on range, alongships- and athwartships-angles, and time [6].

Fish tracks were gridded into 1-m range bins and exported with the summed wedge volume [7] per bin. We calculated fish densities through the summation of fish tracks divided by the respective volume per bin. Probability density functions (PDF) of fish density versus range and depth were generated to identify regions of non-stationary fish densities; regions less than 10 m in range (2.3 m depth) were removed from further analyses due to non-stationary PDF. We divided the remaining data (range 10 m to the seabed) into regions of complete across-channel transects and exported the fish tracks and summed wedge volume for each transect. We estimated the density of corvina (fish 1000 m-3) for each transect by dividing the total number of tracks by the summed wedge volume. Fish densities per transect were mapped in a geographic information system software (ArcMap, Esri, USA) to estimate the spatial distribution of the spawning aggregation by measuring the linear distance of uninterrupted, fish densities per transect greater than 2 fish 1000 m-3.

Mean fish densities per survey were estimated by a transect-volume weighted average of transect densities after an autocorrelation analysis between transects was completed and found to be negligible. Standard errors and 95% confidence intervals of mean survey densities were estimated using bootstrap resampling (n = 10,000). Total corvina abundance per survey was calculated by multiplying estimated fish density per survey by total volume of the survey area. The total volume of the survey area was approximated by integrating the volume of water between the limits of the survey (e.g. first to last transect) based on bathymetric data and a tidal height correction. Standard errors and 95% C.I. of abundance were estimated through the multiplication of bootstrap-estimated values for density with the volume of surveys.

*Passive Acoustic Instrumentation, Sampling, and Analysis*

From the second vessel, we recorded ambient sound at a single random location along each active acoustic transect immediately after the active acoustic vessel passed by, thereby coupling the two measurements in time and space. Recordings were made using a using a calibrated Tascam DR-680 Portable Multitrack Recorder (TEAC Corporation, Japan) and a single HTI-96-MIN hydrophone (High Tech, Inc., USA; sensitivity = -192.0 dBV/µPa). Data were sampled at 192 kHz, digitized with 24-bit resolution, and stored as .wav files on secure digital high capacity (SDHC) memory cards. At each location, 60-second recordings were made with the hydrophone deployed 2 m below the vessel as the vessel drifted across each transect with its engine turned off. Geographic coordinates of each location were saved as waypoints and tracks indexed with time in a handheld GPS.

Individual files were inspected audibly and visually, and 20-second portions of recordings were selected that were free of nearby boat noise and operation disruption, such as segments recorded in air or in the presence of cable strumming. After calibrating the data, sound pressure levels for each location were calculated as root-mean-squared pressure (μParms) and converted to decibels (dBrms *re*:1μPa), where . All calculations were made within Matlab (The Mathworks®, USA). Sound pressure levels were mapped as dBrms and Pa in a geographic information system software (ArcMap, Esri, USA) to estimate the spatial distribution of sound production and the spawning aggregation by measuring the linear distance between uninterrupted, sound pressure levels per transect greater than 150 dBrms. As peak spawning and stable sound production rates occur over a predictable two-hour period [6], cumulative sound exposure levels (SELcum; dB *re*: 1 µPa2-s) throughout the aggregations were calculated by integrating received levels recorded during this period. SELcum values, which estimate the cumulative (additive) sound energy produced during the 2 h spawning event, were used to estimate the potential impact of elevated acoustic exposure on marine life present at the site. Sound pressure levels (dBrms) of the four recordings made outside the spawning period were similarly calculated, and the mean was compared to levels recorded during the spawning period to estimate the magnitude of change in ambient sound attributable to the presence of corvina sound production.

We isolated audio recordings of calls with high signal to noise ratios (where pulses could be identified above background noise) and choruses exceeding 150 dBrms to characterize the individual calls and collective chorusing produced by male corvina. Oscillograms of calls were generated to estimate call duration, pulses per call, pulse duration, pulse interval, and pulse period. We isolated individual pulses from calls to calculate three measurements of received sound pressure levels (e.g., dB measured as 0 to peak (0-p), peak-to-peak (p-p), and rms)and identify the maximum levels recorded that are potentially indicative of source levels (dB at 1 m). We generated pressure spectral density (dB *re*: 1 µPa2/Hz) curves of calls and choruses to estimate their peak frequencies and 3 dB and 6 dB bandwidths (Hz), which describe the distribution of acoustic power as a function of frequency. The mean, 95% confidence intervals (C.I.95), maximum, and minimum values of each measurement were calculated in the linear domain and converted into the logarithm to the base 10 (e.g. dB *re*: 1 µPa) where appropriate.

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