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Sound production at spawning aggregation sites conveys information about the reproductive biology and abundance of fishes

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2018

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UNIVERSITY OF CALIFORNIA SAN DIEGO

Sound production at spawning aggregation sites conveys information about the reproductive biology and abundance of fishes

A dissertation submitted in partial satisfaction of the requirements for the degree
Doctor of Philosophy

in

Marine Biology

by

Timothy James Rowell

Committee in charge:

Marco O. Aburto Oropeza, Chair
Brad Erisman, Co-Chair
Gerald Lynden D'Spain
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2018

The Dissertation of Timothy James Rowell is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

Co-chair

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University of California San Diego

2018

DEDICATION

This dissertation is dedicated to my family.

EPIGRAPH

"…something is happening here
but you don't know what it is…"

Bob Dylan

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

This dissertation was made possible by many people who graciously ensured its completion through assistance, guidance, and support. I would like to thank my committee members who continued to challenge and guide me. Octavio Aburto Oropeza, my advisor, provided me with continuous support to conduct meaningful research. Brad Erisman offered unwavering, selfless guidance through countless discussions to develop the intellectual and scientific merit of my research. David Demer was pivotal in ensuring that I had the best tools and people available to guide me through developing methods in fisheries acoustics; he was a great teacher and mentor. Gerald D'Spain has been a major contributor to my current understanding of ocean acoustics, for that I will always be grateful. Brice Semmens, Jon Shurin, and Phil Hastings have strengthened my skills as a scientist by challenging me to think outside of my comfort zone and apply my research to broader biological concepts. Their willingness to provide insight into topics and themes of research is greatly appreciated.

I would also like to thank everyone who has assisted in my research, notably including B. Erisman, O. Aburto Oropeza, D. Demer, G. D'Spain, B. Semmens, J. Shurin, P. Hastings, J. Hyde, D. Castro-Arvizu, J.J. Cota-Nieto, C. Biggs, B. Martinez-Guerrero, T. TinHan, A. Johnson, A. Ramirez-Valdez, M. Doane, E. Reed, M. Steele, C. López Sagástegui, S. Freeman, J. Montañez Rivera, B. Legare, A. Montes Zamudio, J. Cabrera Rivera, E. Cabrera Rivera, T. Sessions, J. Zwolinski, J. Renfree, S. Mau, staff of Cabo Pulmo Divers, and members of the Aburto Lab, Centro Para la Biodiversidad Marina y Conservación A.C., Gulf of California Marine Program, Comisón Nacional de Áreas Naturales Protegidas (CONANP), and Parque Nacional Cabo Pulmo. This work would not have been possible without the support of the government of Mexico and administrators within CONAPESCA and

CONANP. Funding was provided by grants from the NOAA Advanced Acoustics Program, Walton Family Foundation, David and Lucile Packard Foundation, and World Wildlife Fund Mexico. Lastly, I would like to acknowledge my wife, Sherlynette Pérez Castro, my family, and my friends.

Chapter 1, in full, was submitted for publication as: Rowell, Timothy J.; Aburto-Oropeza, Octavio; Cota-Nieto, Juan José; Steele, Mark A.; Erisman, Brad E. (*in review*) Reproductive behaviour and concurrent sound production of Gulf grouper *Mycteroperca jordani* (Epinephelidae) at a spawning aggregation site. *Journal of Fish Biology*. The dissertation author is the primary investigator and author of this manuscript.

Chapter 2, in part, is currently being prepared for submission for publication of the material. Rowell, Timothy J.; D'Spain, Gerald L.; Aburto-Oropeza, O.; Erisman, B.E. The dissertation author was the primary investigator and author of this material.

Chapter 3, in full, is a reprint of the material as it appears in Biology Letters 2017. Erisman, Brad E.; Rowell, Timothy J. The dissertation author was the primary investigator and author of this material.

Chapter 4, in full, is a reprint of the material as it appears in Scientific Reports 2017. Rowell, Timothy J.; Demer, David A.; Aburto-Oropeza, Octavio; Cota-Nieto, Juan José; Hyde, John R.; Erisman, Brad E. The dissertation author was the primary investigator and author of this material.

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FIELDS OF STUDY

Major Field: Marine Biology

ABSTRACT OF THE DISSERTATION

Sound production at spawning aggregation sites conveys information about the reproductive biology and abundance of fishes

by

Timothy James Rowell

Doctor of Philosophy in Marine Biology

University of California San Diego, 2018

Professor M. Octavio Aburto Oropeza, Chair
Professor Brad E. Erisman, Co-Chair

Sounds produced by fishes during reproductive periods are increasingly being discovered, highlighting the importance of acoustic communication within courtship and spawning behaviors across a diverse assemblage of fishes. While researchers have increased monitoring efforts of fish sounds to infer spatio-temporal patterns of spawning, habitat use, and abundance, a lack of knowledge about the relationships between recorded sounds, reproductive behaviors, and abundance has impeded progress in exposing the utility of fish sounds for understanding the reproductive biology and dynamics of regional populations.

This dissertation assesses the biological importance of sound production in Gulf grouper (*Mycteroperca jordani*) and Gulf corvina (*Cynoscion othonopterus*) and provides evidence that levels of fish sounds are indicative of reproductive activity and abundance. Long-term recordings of Gulf grouper sounds were coupled with observational data and acoustic propagation modeling to describe the mating system and acoustic behaviors of the species. Gulf grouper conformed to a protogynous life strategy and lek mating system with large males that established fixed territories, courted individual females, and pair spawned. Males produced sounds during courtship behaviors and spawning rushes that may have functioned to express fitness to prospective mates; this is the first account of sounds accompanying spawning in groupers. Rates of sound production were statistically related to measures of female abundance and reproductive activity, indicating that fish sounds can be used as estimators of relative reproductive activity and abundance of both sexes outside of observations. Mobile echosounder and passive acoustic surveys of the spawning aggregation site of Gulf corvina documented the sounds, spatial distribution, and abundance of aggregated fish. The site supported up to 1.55 million fish that contributed to a 21-fold increase in ambient noise levels through collective chorusing of the loudest fish sound yet to be documented, warranting future conservation and appreciation of acoustic communication in fishes. A predictive relationship between acoustic levels of chorusing and estimates of density was found, demonstrating that sound levels may be used to estimate the density of soniferous fishes at aggregation sites. In summary, my dissertation advances our understanding of the importance of sounds within reproductive behaviors and identifies future opportunities for improved population monitoring.

INTRODUCTION

Despite being described by Aristotle over two thousand years ago and likely observed throughout history, sound production by fishes has only recently been the focus of published scientific investigations. Starting in the late 19th century, studies on the mechanisms behind fish sound production emerged, identifying the swim bladder and surrounding musculature as a common source of sound generation in many physoclistous species (Dufosse, 1874; Sörensen, 1895; Tower, 1908; Tavolga, 1964). With the development of new recording technologies by the middle of the 20th century, the field of fish bioacoustics greatly expanded with the identification of new species-specific sounds associated with behaviors, such as courtship, spawning, defense, aggression, and feeding (Fish, 1964; Winn, 1964; Fish & Mowbray, 1970). These efforts have continued to the present, resulting in the recognition of sound production in over 100 families of marine fishes (Fish, 1964; Fish & Mowbray, 1970; Hawkins, 1986; Kaatz, 2002; Širović & Demer, 2009; Lobel *et al.*, 2010; Fine & Parmentier, 2015).

While fish sound production occurs across a number of behavioral contexts (Lobel *et al.*, 2010), sounds recorded at fish spawning aggregations (FSAs) that are associated with territoriality, courtship, and spawning hold great promise for increasing our understanding of the reproductive biology of fishes and monitoring of spawning stocks using passive acoustic methods (Rountree *et al.*, 2006; Luczkovich *et al.*, 2008a; Mann *et al.*, 2008). Transient FSAs often occur at predictable times and locations, where conspecific individuals congregate in large numbers for the purpose of reproduction (Domeier, 2012), permitting the rather unique opportunity for populations to be surveyed and studied in a highly synchronized and localized setting (Erisman *et al.*, 2015a). FSAs have long been recognized and exploited by commercial fisheries, yielding large catches with limited effort (Sadovy & Domeier, 2005; Erisman *et al*., 2012). However, fisheries that target FSAs are often unsustainable when improperly managed, resulting in rapid reductions in stock sizes, reproductive output, and in some cases regional aggregation and species extirpations (Sala *et al*., 2001; Aguilar-Perera, 2006; Sadovy de Mitcheson & Erisman, 2012). Since individuals migrate from largely dispersed home ranges to specific locations during brief spawning events (Nemeth, 2009), intense fishing efforts at FSAs can effectively and rapidly remove high percentages of populations normally distributed over large catchment areas, having great implications on the health and trophic structure of adjacent ecosystems (Nemeth, 2012). Consequently, recent support has emerged to increase protections for FSAs and the management of associated fisheries (Russell *et al*., 2012; Erisman *et al*. 2015a; Sadovy de Mitcheson, 2016), but there still remains major gaps in our knowledge of the reproductive biology and requirements associated with FSAs that are necessary for effective conservation (Grüss *et al*., 2014).

The development and expansion of passive acoustic methods to study fishes at FSAs may result in a better understanding of the reproductive biology, acoustic communication, and stock dynamics of vulnerable species and facilitate conservation and restoration efforts. Observations of fish sound production at FSAs have been used to identify the timing and location of spawning and courtship behaviors (Luczkovich *et al*., 1999; Hawkins & Amorim, 2000; Rowe & Hutchings, 2006; Luczkovich *et al*., 2008b; Fudge & Rose, 2009; Walters *et al*., 2009; Locascio & Mann, 2011; Schärer *et al*., 2012b; Rowell *et al*., 2015; Wilson *et al*., 2014). Diel increases in sound production around the known time of spawning support the use of long-term, passive acoustic recordings to not only infer spawning seasonality but also temporal windows (e.g. days, hours) in which reproductive behaviors occur (Connaughton &

Taylor, 1995; Mann *et al*., 2010; Locascio & Mann, 2011; Schärer *et al*., 2012a, 2012b, 2014; Bertucci *et al*., 2015), yielding patterns of spawning activity at high resolution for consideration in future scientific, conservation, and management endeavors. However, in most previous studies, the sounds recorded have only been observed within the behavioral context of males patrolling territories and courtship between a males and females not actively spawning (Lobel *et al*., 2010; Mann *et al*., 2010; Nelson *et al*., 2011; Schärer *et al*., 2012a, 2012b, 2014). While it is likely that increases in courtship behaviors and associated sounds equate to spawning activity as suggested by comparative studies between sound levels and pelagic egg abundance (Luczkovich *et al*., 1999), no studies have directly examined this assumption through visual observation outside of laboratory studies (Montie *et al*., 2017). Thus, new efforts are greatly needed to further document the behaviors associated with sounds recorded at FSAs and determine whether these sounds are indicative of spawning frequency. With such information, recordings of fish sound production could be used to estimate short- and long-term patterns of different behaviors including spawning activity, begin to understanding the function and importance of acoustic communication within reproductive contexts, and identify future threats to acoustic communication and successful reproduction, such as increases in anthropogenic noise.

In addition to understanding the acoustic behaviors of fish within the contexts of biological and behavioral functioning, monitoring and estimating fish abundance and reproductive activity is essential for understanding population dynamics, assessing management strategies, and mitigating the effects of targeted fishing on reproductive output. While initial progress has been made to use received sound levels as indices of relative fish abundance and reproductive activity at FSAs (Luczkovich *et al*., 1999; Gannon & Gannon, 2009; Rowell *et al*., 2012; Rowell *et al*., 2015), difficulties in relating sound production metrics to absolute fish density, abundance, and behaviors have precluded the development of estimation models and the use of passive acoustic methods in population assessments (Rountree *et al*., 2006; Luczkovich *et al*., 2008a; Gannon, 2008; Marques *et al*., 2013). Thus, new efforts that develop and validate the use of fish sounds to quantify fish abundance and behaviors are required to promote incorporation into biological studies, long-term fisheries-independent monitoring, and management plans (Rountree *et al*., 2006; Luczkovich *et al*., 2008a).

With an understanding of rates of fish sound production and sound propagation, it is possible to decipher behavioral activity and estimate abundance from levels of sounds recorded by instrumentation (Rountree *et al*., 2006; Marques *et al*., 2013). However, as rates of fish sound production often vary considerably in time during reproductive periods and are difficult to estimate in-situ (Locascio & Mann, 2008; Mann *et al*., 2010; Schärer *et al*., 2012a, 2014), relating recorded levels of fish sounds to behaviors and abundance has proven challenging. Thus, there is a need increase efforts that seek to understand rates of fish sound production and how they correspond to reproductive activity and abundance. Future efforts that combine acoustic propagation modeling, detection theory, visual observations, and passive acoustic monitoring may facilitate estimates of sound production rates, sequential comparisons with behaviors and abundance, and examinations of acoustic communication capabilities in fishes (Zimmer *et al*., 2008; Helble *et al*., 2013; Marques *et al*., 2013; Johnson *et al*., 2018).

In some fish species, the abundance of sound producers at FSAs produce choruses that prohibit examinations of rates of sound production and comparisons with abundance

(Luczkovich *et al*., 1999; Locascio & Mann, 2011). For such species, relationships between sound levels and density can be modeled through comparisons of sound production levels and independent measures of fish density (Rountree *et al*., 2006). This relatively simplistic approach presents the opportunity to develop species-specific models to assess and monitor spawning stock density, abundance, and biomass from acoustic levels of choruses. Previous works have exemplified the potential of this method through comparisons of sound production indices with CPUE of simultaneous trawls (Gannon & Gannon, 2009), densities of early stage eggs (Luczkovich *et al*., 1999), and relative fish densities estimated with active acoustics (Širović *et al*., 2009) and visual census (Rowell *et al*., 2012), but studies have yet to yield a means to estimate abundance from fish sounds.

The goals of this dissertation were to assess the biological importance of sound production in fishes and examine whether levels and rates of fish sound production are indicative of reproductivity activity and abundance, using Gulf grouper (*Mycteroperca jordani*) and Gulf corvina (*Cynoscion othonopterus*) as case studies. The objectives of Chapter 1 were to describe the mating system and acoustic behaviors of Gulf grouper and identify the importance of acoustic communication within reproductive contexts. The objectives of Chapter 2 were to apply acoustic propagation modeling and detection theory in the interpretation of acoustic recordings and observational data of Gulf grouper, estimate patterns of rates of fish sound production, provide initial information about the communication capabilities of the species, and examine relationships between sound production rates, reproductive behaviors, and abundance. The objectives of Chapter 3 were to describe the sounds and choruses of Gulf corvina, present the impacts of fish chorusing on acoustic environments, and describe the importance of valuing acoustic communication in fishes. The objectives of Chapter 4 were to estimate the abundance and sound levels produced by Gulf corvina and develop predictive models between fish densities and chorusing levels in shallow water environments. The collection of research identifies the importance of sound production within reproductive behaviors and presents new opportunities to monitor fishes through their naturally occurring, acoustic behaviors.

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CHAPTER 1

Reproductive behaviour and concurrent sound production of Gulf grouper *Mycteroperca jordani* (Epinephelidae) at a spawning aggregation site

ABSTRACT

The reproductive and acoustic behaviours of Gulf grouper *Mycteroperca jordani* were studied at a spawning aggregation site in the southern Gulf of California, México. In May 2015 – 2017, divers located and surveyed a spawning aggregation site within Cabo Pulmo National Park. Adult *M. jordani* conformed to a lek mating system in which large males formed territories over sand adjacent to a rocky reef that were spatially segregated from smaller females outside of courtship and spawning periods. Females moved into male territories during evening hours to spawn. Male courtship behaviours targeted a single female, included head shakes and burst rises, and preceded pair spawning prior to sunset. Males and females displayed three shared colour phases, but four phases were sex-specific. During evening hours, courtship, and spawning, both sexes exhibited sexual dichromatism concurrent with reproductive behaviours. The pair-spawning mating system and observations of bimodal size distributions by sex support previous claims of protogyny in the species. Males produced sounds during territorial patrols, courtship, and spawning rushes, which corroborated the importance of acoustic communication within the behavioral repertoire associated with spawning. Long-term acoustic monitoring revealed increases in total sounds detected day^{-1} from March through June with diel increases (*e.g.* evenings) that may be indicative of the spawning season. Observations of spawning on 12 consecutive evenings in May 2017 coupled with extended periods of sound production suggest that spawning does not follow a lunar

rhythm. This first description of the mating system and sounds of the endangered *M. jordani* facilitates future development of seasonal and areal protections to restore and manage the species.

INTRODUCTION

The Gulf grouper *Mycteroperca jordani* (Jenkins & Evermann 1889; Epinephelidae) is a large-bodied top predator endemic to rocky reef habitats from La Jolla, California, USA to Mazatlán, Sinaloa, México with a center of distribution within the Gulf of California (Craig *et al.,* 2011; Figure 1.1). Individuals can grow to total lengths (TL) of 2 m and weights of at least 91 kg (Thomson *et al.*, 2000; Aburto-Oropeza *et al.*, 2008). As the second largest species of grouper in the eastern Pacific, the largest being the Pacific goliath grouper *Epinephelus quinquefasciatus* (Bocourt 1868) (Craig *et al.*, 2011), *M. jordani* was historically the most abundant predatory fish species in the rocky reef ecosystems of Baja California Sur in terms of biomass and subsequently was a productive fishery resource (Sala *et al.*, 2004), generating 45% of total state landings between 1959 and 1960 (Saenz-Arroyo *et al.*, 2005). However, continued intensive fishing led to the collapse of the stock in the 1970s, and the current population size is estimated to represent only 1% of historic levels (Rámirez-Rodriguez, 1996; Saenz-Arroyo *et al.*, 2005). Consequently, *M. jordani* is rarely observed throughout its range (Aburto-Oropeza et al. 2011; Mascareñas-Osorio *et al.*, 2011; Barjau-González *et al.*, 2012; Jorgensen *et al.*, 2016) and contributes to less than 1% of contemporary landings in the southern Gulf of California (Erisman *et al.*, 2010b; Erauskin-Extramiana *et al.*, 2017), which are largely comprised of juveniles (Aburto-Oropeza *et al.*, 2008). The species is currently classified as endangered by the International Union for Conservation of Nature and Natural Resources (IUCN) due to a decreasing population trend and the targeted fishing of vulnerable spawning aggregations (Craig *et al.*, 2008). *M. jordani* is protected in U.S. waters as an endangered species (Federal Register, 2016), but the species remains unregulated in commercial and recreational fisheries of México.

M. jordani is intrinsically vulnerable to fishing pressure due to their long lifespan, late maturation, and aggregate spawning behaviour (Sadovy de Mitcheson *et al.*, 2013). *M. jordani* is estimated to live up to 48.3 years and mature at 97.9 cm based on limited data and anecdotal evidence (Aburto-Oropeza *et al.*, 2008) and likely conforms to a protogynous (female to male sex change) sexual strategy with female-biased sex ratios (Sáenz-Arroyo *et al.*, 2005; Craig *et al.*, 2011), although the sexual pattern of the species has not been confirmed or explicitly examined. The species reportedly forms spawning aggregations around the full moon in the months of May and June based on opportunistic field observations and grey literature (Sala *et al.*, 2003; Sáenz-Arroyo *et al.*, 2005); however, spawning has never been observed. While declines of *M. jordani* populations have been attributed to the overfishing of spawning aggregations (Sáenz-Arroyo *et al.*, 2005), a scientifically supported description of species' mating system and reproductive behaviours, which is essential for the establishment of effective restoration and management strategies (Grüss *et al.*, 2014; Sadovy de Mitcheson, 2016; Erisman *et al.*, 2017), is absent. With knowledge of the spatio-temporal patterns of spawning based on systematic observations and monitoring at aggregation sites (Colin, 1992; Erisman *et al.*, 2007; Colin, 2012), areal and temporal regulations can be designed and implemented to protect spawning aggregations as a means to restore reproductive stocks and regional populations (Nemeth, 2005; Heppell *et al.*, 2012). Identifying the timing and location of spawning and describing the mating system of *M. jordani* is the first pivotal step towards restoring populations of this endangered species.

As fishes produce sounds associated with agonistic, disturbance, and reproductive behaviours (Lobel, 1992; Lobel *et al.*, 2010; Erisman & Rowell, 2017), visual observations coupled with passive acoustic monitoring have increasingly been used to characterize spatio-temporal patterns of habitat use, courtship, spawning, and acoustic behaviours in a number of epinephelid species (Mann *et al.*, 2010; Nelson *et al.*, 2011; Schärer *et al.*, 2012b; Rowell *et al.*, 2015), including the yellowfin grouper *Mycteroperca venenosa* (L. 1758) (Schärer *et al.*, 2012a) and black grouper *Mycteroperca bonaci* (Poey 1860) (Schärer *et al.*, 2014; Locascio & Burton, 2016; Sanchez *et al.*, 2017), which are native to the western Atlantic ocean and the closest living relatives to *M. jordani* (Craig & Hastings, 2007). Direct, repetitive observations of fish behaviours *in-situ* during reproductive periods are often required to describe the mating system of aggregating species (Adreani *et al.*, 2004; Erisman *et al.*, 2007; Adreani & Allen, 2008; Colin, 2012) but are often limited due to personnel, vessel, economic, and visual constraints. The incorporation of acoustic recording devices into visual surveys has identified the inclusion of acoustic communication in reproductive contexts, such as male sound production during courtship associated interactions with females (Mann & Lobel, 1998; Nelson *et al.*, 2011; Bertucci *et al.*, 2015), thereby permitting the monitoring of validated, acoustic behaviours using passive acoustics outside of observational periods (Rountree *et al.*, 2006). With knowledge of the behaviours associated with sound production during spawning aggregations, long-term acoustic monitoring can be used synergistically with visual observations to deduce the daily timing and seasonality of spawning (Schärer *et al.*, 2012a, 2012b, 2014) and estimate abundances remotely from recorded sound metrics (Rowell *et al.*, 2012, 2017). Given the sound producing capabilities of its congeners, complementary descriptions of the acoustic and reproductive behaviours of *M. jordani* would provide a basis to monitor spawning behaviours, seasonality, and relative abundances of spawning stocks at aggregation sites using passive acoustic methods.

The objective of this study was to describe the mating system of *M. jordani* and associated sound production using visual and passive acoustic methods at a spawning site inside Cabo Pulmo National Park (CPNP), Baja California Sur, México. We analyzed three consecutive years of coupled observational-sound data collected during the month of May to describe the reproductive behaviours of *M. jordani* and evaluate the prevalence and variability of sounds produced by males in association with spawning activity. Seasonal, long-term audio recordings were analyzed to describe the sounds and characterize temporal patterns of sound production at the location outside of observational periods. By providing a coupled description of the mating and acoustic behaviours of *M. jordani*, this study will facilitate regional efforts to locate additional aggregation sites and examine spawning dynamics and seasonality using visual and passive acoustic methods. The results of this study and future studies will support the development and assessment of areal and temporal protections established to restore reproductive stocks of the species.

MATERIALS AND METHODS

The study was based on field observations of unhandled fish in their natural habitat and passive acoustic recordings of ambient sound. All methods were conducted in accordance with the guidelines and regulations approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California San Diego under IACUC protocol S13240 and permits issued by SEMARNAT Comisión Nacional de Áreas Naturales Protegidas, Parque Nacional Cabo Pulmo.

STUDY SITE

Observations and acoustic monitoring of *M. jordani* behaviours and sound production took place within the boundaries of CPNP (Figure 1.1). CPNP was established in 1995 with 35% of the 71 km^2 area declared as a no-take reserve, but 100% of the park is locally enforced by the community as a no-take reserve to protect the marine resources and viability of the dive tourism industry (Aburto-Oropeza *et al.*, 2011). The park protects extensive sandy habitats interspersed with outcrops of rocky reefs with low-density coral cover and high fish abundances (Riegl *et al.*, 2007; Egerton *et al.*, 2018). Since its establishment, CPNP has facilitated the recovery of *M. jordani* and other large top predators within its boundaries (Aburto-Oropeza *et al.*, 2011). Due to the large degree of protection coupled with frequent observations of *M. jordani* on reefs within the park (Aburto-Oropeza *et al.*, 2008), as well as unpublished observations of this species in presumed nuptial colouration at this site made decades prior (pers. comm. M.A.S.), CPNP was selected as an appropriate site to locate a spawning aggregation and describe the reproductive and acoustic behaviours of the species. In this study, all observations and acoustic monitoring were made at a single rocky reef and adjacent sandy habitat at a depth of 18 m; the exact location is intentionally omitted to protect the recovering spawning aggregation from potential poaching. The specific location was identified as a potential spawning site prior to the study with the aid of local knowledge about seasonal increases in *M. jordani* abundance (pers. comm. D. Castro-Arvizu).

BEHAVIOURAL OBSERVATIONS AND SOUND IDENTIFICATION

Visual observations and targeted acoustic recordings of *M. jordani* were made at the study site during 30 days of diver (SCUBA) surveys corresponding to 4 days before the full moon (DBFM) to 13 days after the full moon (DAFM) in the month of May in 2015 – 2017.

The month and lunar days of surveys were chosen based on a previous study by Sala *et al.* (2003), who speculated that peak reproductive activity occurs during these periods. As spawning, courtship, and sound production had not previously been observed in the species, 6 days (2 – 8 DAFM) of diver surveys were conducted in mornings (0800 – 1100 hours Mountain Standard Time; MST; UTC - 7) and evenings (1500 – 1900 hours MST) of May 2015 to confirm the presence of a spawning aggregation at the study site and initially document the reproductive and acoustic behaviours of *M. jordani* and diel patterns of courtship and spawning. The results acquired were then used to design subsequent surveys conducted in 2016 and 2017. All times hereafter are referenced to MST. On each day in 2015, two teams of divers completed 2 – 3 roaming surveys in the morning and evening hours across the site and recorded the occurrence, timing, and relative location of individuals, colour patterns, relative sizes, abdominal shape (*i.e.* gravid), behavioural sequences, spawning rushes, and sound production. The sex of individuals was initially identified by distinct behaviours, body sizes, abdominal shape, and colouration, which were later confirmed during observations of gamete release. Observational notes were validated with video data collected with GoPro cameras carried by divers. Sounds captured within video files were extracted to identify the sounds produced by *M. jordani* within specific behavioural contexts.

In May 2016 (7 days; 2 – 8 DAFM) and 2017 (17 days; 4 DBFM – 13 DAFM), two pairs of divers conducted 2 – 3 surveys (n = 99) per evening (1536 – 1911 h) at the same location as 2015. The timing of surveys was selected based on diel patterns of courtship and spawning observed in 2015, which peaked in the hours prior to sunset; spawning and courtship were not observed during morning surveys of 2015. The two survey teams observed a focal individual (male) for the duration of each survey (mean ± 95% C.I. = 22.7 ± 1.7 min) and recorded observations on a customized data collection sheet based on observations made in 2015 (Supporting Information Table S1). Divers carried a GoPro video camera and independent hydrophone (Sound Trap 300 ST; Ocean Instruments NZ; sensitivities = -171.5 & -171.9 dBV μPa^{-1}) attached to a mounting pole to simultaneously record the behaviours and sounds produced by the focal individual. Divers recorded their observations of colouration patterns, location, specific behaviours (see below), and sound production onto data sheets mounted on plastic slates. Male behaviours were ascribed as courtship if they included an interaction with one or more females that resulted in a spawning rush confirmed by the release of gametes. When divers encountered females within the territory of the focal male, their numbers were noted to permit calculations of diver encounter rates along with their colouration, location within the territory, and abdominal shape (*i.e.* gravid). The start and end time of each survey was recorded along with the time of observations and water temperature. Within a given year, each diver observed the same male across all evening surveys, which were identified by distinct scarring patterns, caudal fin shape, and territory location. Observations of spawning by other fish species present at the site were also recorded.

Observations from 2016 and 2017 were validated with the video and audio data collected during evening surveys. Video files were temporally synchronized with hydrophone recordings within a commercial editing software (Final Cut Pro X, Apple) and exported for analysis. Behavioural observations were verified and collectively used to describe the mating system and associated behaviours of male and female *M. jordani*. Diel patterns of courtship and spawning events were examined as proportions within hour bins (1500 – 1900 hours) after being weighted for observation time. Differences in hourly medians were tested with a nonparametric Kruskal-Wallis test ($\alpha = 0.05$), because data did not conform to the assumptions of parametric testing. Audio files were examined audibly and visually within spectrograms to corroborate the prevalence and variability of sounds produced by males during territorial patrols, aggression, courtship, and spawning rushes. Once identified, specific sounds were characterized based on their temporal and spectral properties and enumerated for each behaviour, yielding a catalogue of sounds produced by males in different behavioural contexts. Examples of specific types of sounds were isolated and extracted to facilitate future identification in data generated during long-term acoustic monitoring.

Terminology and descriptions of behaviours associated with territorial patrols, aggression, courtship, and spawning rushes followed those reported for other species of epinephelids with some species-specific variation (Gilmore & Jones, 1992; Samoilys & Squire, 1994; Donaldson, 1995; Zabala *et al.*, 1997; Erisman *et al.*, 2007; Erisman *et al.*, 2010a; Schärer *et al.*, 2012a, 2014). Behaviours included patrols, lateral standoffs, chases, head shakes, burst rises, and spawning rushes (Table 1.1). *Patrol* = male swims rapidly along territorial boundaries and interiors over sand. When males encounter each other along territorial boundaries they engage in *lateral standoffs*, which involves both individuals swimming jointly along the boundary before dispersing inward towards their respective territories. *Chases* = intruding male is driven out of another male territory at a rapid speed. Courtship of females includes: *head shake* = a male approaches a female in an encircling pattern, shakes the anterior portion of his body and head, and departs from the female in an upward arching movement, and *burst rise* = a male swims along the bottom and rises vertically to the mid-water column in an arching pattern over a single female. *Spawning rush* = a male and female rise in the water column at opposing angles and then unite as a pair, spiral upward, release gametes, and return to the bottom.

ESTIMATES OF MALE NUMBERS AND TERRITORIES

During the evenings (1800 – 1900 hours) of May 2016 and 2017 (16 days, 2 DBFM – 12 DAFM), additional transect-video surveys (n = 16) were conducted along the sand-reef boundary of the study site to enumerate the males present at the study site, based on unique body markings, and estimate the distribution of male territories. Upon encountering males, divers observed each individual, noted distinguishing scars and caudal fin shapes, and identified submersed landmarks at the estimated center locations and boundaries of male territories in each day. Video data were examined to confirm the presence and location of each male as described by diver notes. Attempts to identify individual males that were present in both 2016 and 2017 were made from video data. Data from each year were used to determine whether males maintained fixed territories throughout each observational period. The distribution of individual male territories in 2017 surveys was compared to their distributions in morning hours (see below). After documenting the persistence of individual males at established locations throughout evening surveys in 2017, divers released surface marker buoys at the estimated center locations and boundaries of male territories within the survey area. The locations of each buoy deployment were immediately recorded with a handheld GPS (GPSMap 78; Garmin) by the captain of the dive vessel. Territories in 2017 were mapped for each male based on GPS locations and *in-situ* observations. Territories in 2016 were not mapped with geographic coordinates.

ADDITIONAL MORNING OBSERVATIONS AND FISH LENGTHS

Additional morning surveys (0800 – 1100 hours; n = 26) were conducted by divers in May 2017 (13 days, 2 DBFM – 10 DAFM) to characterize the colouration patterns, spatial distributions, and size ranges of males and females during the non-spawning period. The colouration of males and females encountered and their location relative to the reef and sand habitats were documented on dive slates and recorded with GoPro cameras. The locations of males were compared to their territorial distributions in evening surveys. The total lengths (TL) of males and females were estimated with a custom machined parallel laser-camera unit. The unit consisted of a GoPro camera configured for narrow view recording and two parallel lasers spaced 10 cm apart. The system was calibrated on land prior to each survey following the methods described by Heppell *et al.* (2012). Upon encountering confirmed males and gravid females, individuals were recorded with the lasers perpendicular to lateral side of the fish. From the video, still images were captured of fish when laser markings were clearly visible and perpendicular to the fish. Images were imported into Image J image analysis software, and the TL of fish estimated using the "Set Scale" and "Measure" functions (Heppell *et al.*, 2012). The bias of measurements was assumed to be equal across all estimations but was not evaluated. Size ranges and mean TL for each sex were estimated. While individual males could be identified in 2017 for sizing, individual females could not be distinguished in the study and pseudoreplication likely occurred and influenced estimates of mean female TL. Thus, size ranges of males and females were more appropriately targeted in this study.

SOUND CHARACTERIZATION AND LONG-TERM MONITORING

In order to characterize the sounds produced by *M. jordani* and examine temporal patterns in acoustic behaviours, long-term acoustic monitoring at the site occurred over the three-year, seasonal study. Ambient noise was recorded with a DSG-ST (Loggerhead Instruments; sensitivity = -168 dBV μPa^{-1}) and two SoundTrap 300 STD (Ocean Instruments NZ; sensitivities = -175.5 & -171.9 dBV μPa^{-1}) long-term, single hydrophone, underwater acoustic recorders, which were deployed for discrete durations at a fixed location at the site. A single recorder was present at the site throughout each season, and instruments were replaced in May due to battery and memory constraints. The passive acoustic monitoring station was approximately 15 m from the reef and at the boundary between two male territories. The recorder was attached to a stainless steel rod and cement base 1 m above the seafloor at a depth of 17 m. Ambient noise was recorded for a period of 2 min every 10 min from 9 March – 28 June 2015, and 1 min every 5 min from 22 February – 14 July 2016 and 11 February – 31 August 2017. Files were digitized at a sample rate of 96 kHz in 2015 and 48 kHz in 2016 and 2017. Upon retrieval, audio files were extracted and saved as .wav files for further analysis. Bottom temperature was recorded with TidbiT sensors (Onset Computer Corporation) and calibrated sensors within the SoundTrap 300 STD units during the recording periods.

Two types of *M. jordani* sounds (*e.g.* short and long variations) with high signal to noise ratios (see Results) were identified and extracted from the long-term dataset for characterization. Pulse and tonal components of each sound type were analyzed separately. Oscillograms of sounds were generated to estimate the number of pulses $sound^{-1}$, pulse duration, interpulse duration, and duration of tonal (harmonic) components. Pressure spectral density level (dB re: 1 $\mu Pa^2\ Hz^{-1}$, 1 Hz resolution) curves of pulses and tonal parts were produced to estimate their dominant frequencies and 3 dB and 6 dB bandwidths. A total of 204 sounds were used for characterization. Oscillograms and spectrograms (Kaiser window, FFT length = 8192 points) of sound types and combinations that were observed during visual surveys were created in Matlab (The Mathworks). Source levels (dB_{rms} re: 1 μPa at 1 m) of sounds were estimated from recordings in which males were observed producing sounds 1 m from the fixed hydrophone during diver surveys and additional occurrences of sounds produced at a distance from the hydrophone estimated by divers that were corrected for transmission loss (dB) using a spherical spreading approach. Estimated distance from the hydrophone for all sounds was verified in video recordings.

TEMPORAL PATTERNS OF SOUND PRODUCTION

Files resulting from the three years of long-term acoustic monitoring were analyzed manually from spectrograms generated in Matlab (Kaiser window, FFT length = 65536 points for 2015 data, FFT length = 16384 points for 2016-2017 data) to examine temporal patterns in sound production. Each file was visually inspected, and the number of *M. jordani* sounds file^{-1} was recorded. Sounds were summed day^{-1} and multiplied by five to account for sampling effort and estimate the total sounds detected day^{-1}. Time series of total sounds day^{-1} were generated to visualize patterns in daily sound production for the duration of the recording period. To evaluate seasonal patterns of sound production, mean sounds day^{-1} were compared between months for each year and all years combined. During peak lunar months of recorded sounds (*e.g.* April and May), total sounds day^{-1} were analyzed in relation to DAFM (full moon = 0 DAFM) to assess the presence of lunar patterns in sound production. Spectral analyses of daily totals in peak lunar months were conducted in Matlab to assess the periodicity of recorded sounds for each year of monitoring. Diel patterns of sound production were estimated by calculating the proportion of recorded sounds within hour bins. Data were tested for differences in monthly, daily, and hourly patterns of total sounds with nonparametric Kruskal-Wallis tests ($\alpha = 0.05$).

RESULTS

BEHAVIOURAL OBSERVATIONS

In May 2015, a spawning aggregation of *M. jordani* within CPNP was confirmed with observations of courtship, gamete release, and sound production. During observational periods in 2016 and 2017, individual males, identified by unique body markings and released gametes, formed discrete territories over sandy habitats abutting the rocky reef, and individuals were observed to maintain the same territories throughout each field season (Figure 1.2). Males were present within their respective territories during morning and evening surveys in 2017. The territories of individual males in 2017 measured approximately 40 – 50 m in total length along the reef tract based on estimated center and boundary locations; territories extended approximately an equal distance seaward over the sand (Figure 1.2). Territories of males bounded each other, but did not conspicuously overlap in space. A total of five males were observed within the survey area (230 m longitudinally) in 2016 and 2017; two additional males were observed south of the study site during opportunistic surveys in 2017. However, due to different scarring patterns observed across years, it is uncertain whether the same individual males were present at the site in both years. During morning hours (0800 – 1100 hours) in 2017, males were present within their respective evening territories in close proximity to the edge of the reef and swimming slowly. Females, identified by gravid abdomens, in morning surveys were observed over the interior of the reef tract. Spatial separation of sexes during morning surveys was persistently observed; however, on three occasions in 2017, males were seen circling individual females and displaying the head shake behaviour within 1 m of females that was accompanied by a sound, a behaviour commonly observed during evening surveys.

During evening surveys (1536 – 1911 h), males were more active, as evidenced by their constant patrolling along the boundaries and interiors of their territories over sand within a few meters of the bottom (Table 1.1). During patrols, males occasionally rose off the seafloor along the reef edge before returning to the described territorial patrols. During early evening surveys (1500 – 1600 h), females remained over the reef and were not commonly encountered by divers within male territories (encounter rate = 2 females hr^{-1}) but sequentially were observed at a higher frequency within male territories over sand from 1600 – 1800 h (encounter rate = 34 females hr^{-1}). Fewer females were observed within male territories after 1800 h (encounter rate = 5 females hr^{-1}). Females moved among territories with no apparent fidelity to a single male, although individual females were not followed to quantify the number of males encountered.

Males patrolled and defended territories during evening observations (1536 – 1911 h), although most activity ceased after 1830 h. The defense of male territories was observed on 17 occasions during evening surveys in 2016 – 2017 and included lateral standoffs and chases (Table 1.1). During lateral standoffs, males with adjacent territories approached each other along their territorial boundaries and jointly swam laterally before dispersing (n = 12). During these exchanges, males oriented themselves to display their dorsal markings towards the competitor. During other interactions, males actively chased intruding males out of their territories (n = 5). On all chases, males were successful in removing the intruder male from their territories. Sounds were not identified during recordings of male-male interactions. On one occasion in 2017, a male chased a Bull Shark (*Carcharhinus leucas*), which were present at the study site during observations, out of his territory. Males were also observed chasing Yellowfin Surgeonfish (*Acanthurus xanthopterus*) and mobula rays (*Mobula sp.*) out of territories.

Males actively engaged females within their territories during evening hours (1500 – 1900 hours), displaying a series of courtship events that resulted in pair spawning (Table 1.1; Figure 1.3). Upon encountering females within the lower one-third of the water column during territorial patrols, males would target a single female and initiate a spawning bout. Courtship behaviours that preceded spawning included: (1) head shakes and (2) burst rises. Of the 475 observed courtship behaviours, 16% (n = 77) were head shakes, which occurred when a male encircled a female, either on the seafloor or in the water column, shook his head and anterior portion of his body within 1 m of the female, and then moved away from the female in an overarching vertical rise (Figure 1.3). The remaining 84% of courtship observations (n = 398) were burst rises; males swam over the seafloor and rose vertically to the mid-water column in an arching pattern over a female in the lower water column (Figure 1.3). Head shakes preceded burst rises but were not an obligatory antecedent to burst rises. Following both courtship behaviours, males either returned to patrolling their territories or swam in a circle path along the bottom before returning to a female for further courtship or to spawn.

A total of 134 spawning rushes were observed over the three-year study period, with 94 rushes resulting in confirmed gamete release. Days of spawning observations corresponded to 2 DBFM and 2 – 13 DAFM. Males and females initiated pair spawning rushes by vertically rising at an angle opposite to each other and uniting mid-water (Figure 1.3; Table 1.1). Once united, the male and female spiraled upward with their heads adjoined, and gametes were released during upward thrusts approximately 2 m from the surface. After spawning, the male and female returned to the seafloor separately or with the male chasing the female downward. While only pair spawning was observed, additional females, when present, rose vertically in the vicinity of the pair after gamete release but did not enter the gamete cloud or release gametes. Subsequent male spawning with these females was not confirmed. Individual males spawned up to 11 times within a survey (37 min; ~18 spawns hr^{-1}) and with multiple females based on observations of different female sizes; however, individual females were not discernible given the focal-male methods of this study. The mean (± 95% C.I.) number of spawns observed during surveys of an individual male was 2 ± 1 spawns hr^{-1}. All observations of courtship and spawning events occurred between 1500 and 1900 h, peaking in the 1600 and 1700 hours (Figure 1.4). The hourly medians of events, weighted for observation time, were significantly different (Kruskal-Wallis test, $H(4) = 17.2$, $p = 0.002$). Spawning was observed between 1643 and 1840 h during the study period with 2%, 90%, and 8% of spawning rushes occurring in the 1600, 1700, and 1800 hours, respectively. Sunset during May surveys occurred between 1847 and 1859 h. Mean daily water temperatures were 22.42 – 24.32 °C on days with spawning.

FISH LENGTHS AND COLOURTATION

The confirmation of sexes through observations of milt by the larger individual within a spawning pair permitted the sizing and documentation of temporal patterns of colouration in males and females located within the survey area throughout the study. Individual males in

2017 ranged in TL from 133.7 cm to 149.5 cm (mean ± 95% C.I. = 140.4 ± 6.4 cm, n = 5), while TL of gravid females ranged from 72.6 cm to 125.4 cm (mean ± 95% C.I. = 102.6 ± 4.5 cm, n = 33).

Seven colour phases were observed: mottled, camouflage, dark, gray-head, silver, white-back, and white-belly (Figure 1.5). Mottled phase was observed during morning surveys and occurred in both males and females. Mottled phase was typically observed outside of spawning periods and was not associated with courtship and spawning. Camouflage phase occurred in both male and females when individuals were resting stationary on the seafloor over reef habitat; it was not observed during evening hours. Dark phase was observed in both males and females when either swimming or suspended in the water column over reef habitats in morning and evening hours. Dark phase was not observed during courtship or spawning. Gray-head phase was only observed in males and consisted of a gray head and silver body with dark fin edges. Gray-head phase was observed during morning surveys and evening surveys when males were not patrolling territories, courting females, or spawning. The uniform silver phase was only present in females that were swimming over sandy habitats. Females exhibited the silver phase during morning and evening hours when not interacting with males. White-back phase consisted of a gray body, white fins with dark borders, and a conspicuous white saddle encompassing the dorsal region; it was only observed in males during territorial patrolling, aggression, courtship, and spawning. While most commonly observed in the evening, males also exhibited this colour phase during interactions with females during morning surveys. White-belly phase occurred only in gravid females during evening hours after migrating from the reef to male territories over sand and was exhibited during courtship and spawning interactions with males. White-belly phase included patterns of dark blotches over the body and a white abdomen; dark blotches were intensified in terms of contrast with the surrounding colouration during courtship and spawning. The white-belly phase was indicative of females in spawning condition in this study.

SOUND IDENTIFICATION AND CHARACTERIZATION

Sound production by male *M. jordani* was first identified during evening surveys on 5 May 2015 and recorded by divers within the contexts of territorial patrols, courtship, and spawning events throughout the study. We observed no instances of sounds produced by females. Of the sounds recorded during diver surveys (n = 545), 7.5% were recorded when males were alone and 92.5% when interacting with females. During courtship displays, 97% of head shake behaviours were accompanied by recorded sounds, while 80% of burst rises were associated with sounds. Sounds were produced by males during every spawning rush recorded with audio and video (100%) prior to uniting with the female and gamete release. Males also produced sounds after gamete release during male-female chases, which occurred in 25% of observed spawning rushes. During surveys, males produced two types of sounds: (1) short and (2) long tonal sounds, as well as multiple combinations (Figure 1.6). Long and short variations accounted for 72% and 28% of the sounds recorded during surveys, respectively. Sound variations and combinations were observed across all courtship and spawning behaviours; thus, they were not unique to specific behaviours. Long and short sounds were designated by differences in the duration of tonal (harmonic) components that were preceded and followed by a variable number of short, low frequency, repeated pulses (Figure 1.6; Table 1.2). The tonal part of long sounds lasted 2394 ms on average and had a mean fundamental frequency of 49.8 Hz (n = 162), while the tonal part of short sounds had an average duration of 666 ms and a fundamental frequency of 52.3 Hz ($n = 42$). During diver surveys in 2017, males were observed producing long sounds during courtship with a female within 1 m from the long-term recorder ($n = 3$) and an additional 15 sounds (long = 12; short = 3) were produced within 3 – 8 m of the recorder. Estimated source levels of the 15 recordings of long sounds ranged from 132.5 $\mathrm{dB_{rms}}$ to 143.9 $\mathrm{dB_{rms}}$ (mean ± 95% C.I. = 138.5 ± 1.8 $\mathrm{dB_{rms}}$ re: 1 μPa). Estimated source levels of the 3 recordings of short sounds ranged from 130.6 $\mathrm{dB_{rms}}$ to 135.2 $\mathrm{dB_{rms}}$ (mean ± 95% C.I. = 132.3 ± 2.8 $\mathrm{dB_{rms}}$ re: 1 μPa).

TEMPORAL PATTERNS OF SOUND PRODUCTION

M. jordani sounds were recorded throughout the duration of long-term monitoring with daily totals varying among days and months (Figure 1.7). In 2015 and 2016, mean sounds day^{-1} were highest in the months of April and May, exceeding an average of 200 sounds day^{-1}, while means exceeded 200 sounds day^{-1} in the months of April – June 2017 (Figure 1.8). Across all three years, recorded sounds increased from February to March, peaked during April and May, and decreased thereafter. The differences between monthly medians were significant for each year (Kruskal-Wallis test, 2015: $H(3) = 59.0$, $p < 0.001$; 2016: $H(5) = 47.8$, $p < 0.001$; 2017: $H(6) = 147.1$, $p < 0.001$). Daily totals of recorded sounds were sporadic in the peak lunar months of April and May, and spectral analyses revealed no apparent lunar rhythm in relation to sound production (Figure 1.9). Medians for DAFM were not significantly different for the lunar months of April and May (Kruskal-Wallis test, $H(28) = 16.3$, $p = 0.96$). Sounds were recorded in all hours, but a diel trend was observed with the highest proportions occurring prior to sunset within the 1600 and 1700 hours (Figure 1.4).

The hourly medians of sounds were significantly different (Kruskal-Wallis test, H(23) = 2202.6, $p < 0.001$).

NOTES ON SPAWNING IN OTHER SPECIES

Group spawning of Pacific creolefish *Paranthias colonus* (Valenciennes 1846) and leopard grouper *Mycteroperca rosacea* (Streets 1877) was opportunistically observed at the study site during surveys. *P. colonus* spawned in groups of 12 to 34 fish with multiple group rushes occurring throughout the aggregation during brief periods of observation (Sala *et al.*, 2003). Spawning was observed in May 2016 and May 2017 (4 days; 3, 6, 12, 13 DAFM) from 1635 to 1720 h. All events occurred over the interior of the reef in the water column. *M. rosacea* were not aggregated at the study site during May 2016 but were present in high abundances over reef habitat at the site in May 2017. Spawning was observed on six evenings (2, 4 – 8 DAFM) and occurred from 1743 to 1848 h. Group spawning included 10 to 17 individuals and followed patterns described by Erisman *et al*. (2007). As events in both species occurred during the spawning period of *M. jordani*, the study location represents a multi-species spawning aggregation site.

DISCUSSION

COURTSHIP AND SPAWNING BEHAVIOUR

This study provides the first description of the mating system and acoustic behaviours of *M. jordani* made through repeated observations of courtship and spawning at the only confirmed, modern-day spawning aggregation that forms within the protective boundaries of CPNP. Based on observations, *M. jordani* conformed to a lek (Bradbury, 1981), pair spawning mating system. Males provided no parental care and formed large discrete territories within an arena over sand habitat that provided no beneficial resources (Bradbury, 1981). Males maintained and defended the same territories during morning and evening surveys within survey periods, indicating a high level of site fidelity that is characteristic for territorial pair spawners seeking to maximize encounter rates with females (Petersen, 1988; Gladstone, 1994; King & Withler, 2005). Outside of evening hours, females were positioned over the reef and were spatially segregated from male territories, which may be a behavioural adaptation to avoid male harassment (Ruckstuhl & Neuhaus, 2005; Darden & Croft, 2008). In accordance with a lek, females progressively relocated to male territories prior to sunset to engage in courtship and spawning (Warner, 1990; Gladstone, 1994; Luckhurst, 2010). Observations of individual females moving among male territories suggests that females select mates based on attributes yet to be fully understood, but selections may be influenced by assessments of male courtship displays (Kodric-Brown, 1993; Sargent *et al.*, 1998). When present, males courted females with head shake and burst rise displays that included sounds and preceded pair spawning, but success rates of post-courtship spawning were not assessed in this study.

Courtship and spawning events occurred between a single male and female and were accompanied by male sound production. Spawning bouts, which were initiated by courtship displays and terminated with spawning, were observed to commence 2-3 hrs prior to sunset and co-occurred with the arrival of females to male territories as detected by diver encounter rates. While surveys of focal males were not conducted during morning hours, the spatial segregation of sexes, colour patterns, limited interactions between sexes, and no observations of spawning during morning surveys suggest that spawning is limited to the hours prior to sunset. Two distinct courtship behaviours, head shakes and burst rises, were observed throughout evening diver surveys. Burst rises occurred more often and are thought to be an important antecedent to successful vertical spawning rushes as reported in other reef fishes (Colin *et al.*, 1987; Zabala *et al.*, 1997; Adreani *et al.*, 2008). Encirclement with head shakes, while less commonly observed in this study, is a common courtship behaviour of other species of epinephelids (Gilmore & Jones, 1992; Donaldson, 1995; Samoilys & Squire, 1994; Erisman *et al.*, 2010a; Kline *et al.*, 2011; Schärer *et al.*, 2012a, 2014) and may facilitate the positioning of females higher in the water column for subsequent courtship and spawning rushes (Zabala *et al.*, 1997; Whaylen *et al.*, 2004). These two courtship displays may enable males to establish dominance and express fitness to females (Kodric-Brown, 1993; Barbosa & Magurran, 2006; Weir & Grant, 2010).

Pair spawning was exclusively observed, and males spawned with multiple females of different sizes during most surveys when spawning was witnessed. We were unable to assess whether females spawned more than once within days or survey seasons; however, daily spawning has been observed in other species of epinephelids (Samoilys & Squire, 1994; Rhodes & Sadovy, 2002; Erisman *et al.*, 2007). Peak spawning occurred 1 – 2 hours before sunset and was rarely observed around the time of sunset. However, as the sun sets behind a mountain range at the site, light levels may have influenced the timing of spawning and in part explain why peak activity occurred earlier than some other species of epinephelids that are reported to spawn at sunset (Erisman *et al.*, 2007; Schärer *et al.*, 2012a).

Pair spawning behaviours of *M. jordani* align with the behaviours described for closely related *M. bonaci* (Craig & Hastings, 2007; Heyman & Kjerfve, 2008). Similar to *M. bonaci*, male and female *M. jordani* united in the water column and spiraled upward before releasing gametes. However, observations of gamete release approximately 2 m below the surface diverges from *M. bonaci* that have been observed spawning at deeper depths (Heyman & Kjerfve, 2008) and may have been influenced by the shallow depths at the site (18 m). Observations of additional females rising to spawning pairs after gamete release did not support the potential for group spawning at the site but may have permitted further assessments of male fitness and mate selections of other females (Dugatkin, 1992). However, given the extensive estimated decline in population sizes since the 1960s (Saenz-Arroyo *et al.*, 2005) coupled with the density dependence of pair versus group spawning in some protogynous species (Warner & Hoffman, 1980b; Warner, 1982), the mating system of *M. jordani* may be plastic and capable of shifting towards group spawning in high density aggregations with sperm competition (Warner, 1984; Erisman *et al.*, 2009).

FISH LENGTHS AND EVIDENCE OF PROTOGYNY

Observed pair spawning coupled with discrete and bimodal length distributions by sex agrees with previous studies that describe the reproductive strategy and length distributions of other territorial, protogynous species with low sperm competition (Warner, 1984; Domeier & Colin, 1997; Sadovy de Mitcheson & Liu, 2008; Erisman *et al.*, 2009). Thus, this study supports previous claims that *M. jordani* follows a protogynous life strategy (Sáenz-Arroyo *et al.*, 2005; Erisman *et al.*, 2009; Craig *et al.*, 2011), but histological analyses are needed to confirm protogyny (Coleman *et al.*, 1996; Erisman *et al.*, 2010a). While bimodal length distributions were present at the site, it is likely that size ranges may overlap at more populated aggregation sites or with more samples (Whiteman *et al.*, 2005; Erisman *et al.*, 2010a). The smallest gravid female we observed was 72 cm TL, which is smaller than the previous estimate for length of sexual maturity (*e.g.* 97 cm, Aburto-Oropeza *et al.*, 2008). The smallest male observed at the site was 133 cm TL in contrast 125 cm TL estimated for the largest female, providing an initial estimate of potential length range at sex change, but additional studies are needed to understand the population structure. Given that shifts in population structure can occur in suppressed, low-density populations (Lutnesky, 1994; Olsen *et al.*, 2005), length of maturation and sex change may exhibit plasticity in response to competition and available reproductive roles (Warner, 1984; Sadovy & Liu, 2008; Erisman *et al.*, 2009). As regional abundances of *M. jordani* are considered to be a fraction of 1940 levels (Rámeriz-Rodriguez, 1996; Saenz-Arroyo *et al.*, 2005) and the annual number of males present within the survey area minimal (*e.g.* 5 males), the size ranges observed may differ from those found in regions with higher abundances and prior to the collapse of the stock in the 1970s.

COLOURATION

Differences in colour patterns between morning and evening surveys indicated the presence of specific colours associated with courtship and spawning for each sex. While mottled, camouflage, and dark phases were common among males and females, sexual colour dimorphism was evident for what we describe as gray-head, silver, white-back, and white-belly, with the latter two being reproductive colourations for males and females, respectively. Males typically displayed the gray-head phase during morning hours that incorporated light posterior colouration with dark fin edges and an anterior “sunburst” design similar to *M. bonaci* (Heyman & Kjerfve, 2008; Luckhurst, 2010). Silver phase of females was uniformly light gray and lacked the dark gray head exhibited by males. During evening surveys when males were patrolling territories, courting, and spawning, they developed a more uninform dark gray colouration with a contrasting white dorsal saddle (white-back), previously described by Sala *et al.* (2003), that may convey dominance to competing males and fitness to potential mates (Gilmore & Jones, 1992; Sargent *et al.*, 1998). In evening hours, gravid females displayed the white-belly phase upon migrating to male territories that included a highly decorative blotching pattern from head to caudal fin and a white abdomen. The white-belly phase likely communicates a readiness to spawn and was maintained during courtship and spawning events with males. Sexual colour dimorphism in *M. jordani* corroborates a pattern of colour differences in epinephelids (Johannes *et al.*, 1999; Tuz-Sulub *et al.*, 2006; Erisman *et al.*, 2010a; Kline *et al.*, 2011) and support future assignments of sex from white-back and white-belly colourations during the evening hours of spawning periods.

SOUND PRODUCTION AND ACOUSTIC COMMUNICATION

Males produced two main types of species-specific sounds (*i.e.* long and short variations) that were produced singularly or in combination (Figure 1.6) during patrols, courtship, and spawning rushes but were not associated with a specific behaviour. Sound variability during courtship behaviours has previously been documented in other epinephelids, such as Atlantic goliath grouper *Epinephelus itajara* (Lichtenstein 1822), red grouper *Epinephelus morio* (Valenciennes 1828), and red hind *Epinephelus guttatus* (L. 1758) that produce long, short, and combination sounds at spawning aggregations (Mann *et al.*, 2009, 2010; Nelson *et al.*, 2011), but explanations for variations have yet to be evaluated. The sounds of *M. jordani* contain tonal parts with harmonic components that overlap in frequency and estimated source levels of sounds produced by other *Mycteroperca* spp. (Schärer *et al.*, 2012b, 2014). Fundamental frequencies were on the order of 50 Hz for both types of sounds and may be physiologically driven by the size of males, as large epinephelids tend to produce sounds of lower frequency (Mann *et al.*, 2009; Schärer *et al.*, 2014; Bertucci *et al.*, 2015) when compared to smaller-bodied species (Nelson *et al.*, 2011; Mann *et al.*, 2010).

The inclusion of sounds produced by males during nearly all courtship and spawning events corroborates the importance of acoustic communication within reproductive contexts (Lobel *et al.*, 2010; Vasconcelos *et al.*, 2011; Amorim *et al.*, 2015). Male *M. jordani* produced sounds when patrolling territories in the absence of females, which may function to attract females (Lobel *et al.*, 2010), but acoustic behaviours were observed more often upon the arrival of females to territories in the late evening hours when courtship and spawning behaviours occurred. Males combined visual courtship behaviours with acoustic signaling when directly interacting with females in nearly all observations, but we may have underestimated the prevalence of sounds during courtship due to acoustic interference with bubble noise and the distance of some observations. Importantly, all spawning rushes that were observed included male sound production, indicating for the first time in groupers, to the best of our knowledge, that sounds are not only associated with courtship but also with spawning rushes. Previous works have ascribed sounds produced by groupers as courtship associated, because spawning was unable to be observed or concurrent sound recorded (Mann *et al.*, 2010; Nelson *et al.*, 2011; Schärer *et al.*, 2012a, 2012b, 2014). However, within some studies, levels of sound production increased during hypothesized times of spawning. Thus, given the results of this study and previous observations of temporal patterns of sound production, it is probable that other species produce sounds during spawning rushes that have yet to be documented.

Sound production may facilitate attraction, courtship, and spawning when visual cues are not possible due to turbid waters or nocturnal reproduction (Erisman & Rowell, 2017; Koenig *et al.*, 2017; Rice *et al.*, 2017). However, this study corroborates findings that acoustic and visual behaviours are tightly linked in a number of species and may serve to express male fitness to females and increase reproductive success (Rowe & Hutchings, 2004; Lobel *et al.*, 2010; Vasconcelos *et al.*, 2011; Amorim *et al.*, 2015). Tonal sounds are generated by fast contractions of specialized muscles that are energetically demanding (Rome *et al.*, 1996; Rome, 2006); therefore, physically fit males may be better suited to communicate vigor to females (Myrberg *et al.*, 1993). Acoustic signaling can also be used to convey perceived dominance as a function of size, as larger fish within the same species have been shown to produce lower frequencies of sound (Myrberg *et al.*, 1993; Malavasi *et al.*, 2003) that attract more females (Myrberg *et al.*, 1986), but further work is needed to determine the importance of frequency in female mate selection (Lobel *et al.*, 2010). Sound production of *M. jordani* is clearly an important component of the mating system, and variability in sound properties and production rates may be associated with future reproductive success.

TEMPORAL PATTERNS OF SOUND PRODUCTION

Detections of species-specific sounds throughout entire sampling periods indicate the presence of at least one male on any given day when at least one sound was recorded but do not demonstrate that courtship and spawning occurred over the same duration nor reflect changes in fish abundance given the limitations of our methods (Rowell *et al.*, 2012, 2017). The sounds described in this study proportionally were observed most often during interactions with females that often resulted in spawning. However, considering that visual observations were only made for short periods of time during the month of May each year and males were also observed to generate sounds in the absence of females when patrolling territories, the sounds described may also be produced within additional behavioural contexts, such as defense of non-spawning territories or agonism (Mann & Lobel, 1998; Vasconcelos *et al.*, 2010; Tricas & Boyle, 2014). Despite the limitations of our current understanding, the highest proportions of sounds recorded occurred over a two to three-month period during each year with inter-annual variability, which conforms to the duration of spawning seasonality and elevated sound production in other species of epinephelids (Domeier & Colin, 1997; Nemeth, 2005; Erisman *et al.*, 2007; Rowell *et al.*, 2012). Mean sounds day^{-1} were highest from the months of March to June but peaked on average during the months of April and May. Based on the strong association of sounds with courtship and spawning in May and previous evidence of spawning seasons lasting two to three months for epinephelids in the Atlantic and Gulf of California (Colin *et al.*, 1987; Erisman *et al.*, 2007), we speculate that the spawning season of *M. jordani* occurs from March to June with annual shifts of plus or minus a month. However, future studies are needed to test this hypothesis. Peak months were preceded and followed by a buildup and falloff of sounds detected, respectively, in accordance with patterns observed in other species of epinephelids (Schärer *et al.*, 2012b; Rowell *et al.*, 2015). Outside of peak months when sounds were still detected, males may have arrived to the site prior to females and spawning activity to establish territories and dominance (Rhodes & Sadovy, 2002; Nemeth *et al.*, 2007), but future efforts are needed to assess pre-spawning dynamics.

Sound production did not exhibit patterns in relation to lunar phase, as has been described in a number of related species (Schärer *et al.*, 2012a; Rowell *et al.*, 2015), indicating that courtship and spawning may occur continuously during the reproductive season and are not driven by lunar forces. This claim is supported by observations of spawning occurring from 2 DBFM to 13 DAFM, a duration that exceeds the number of days typical for epinephelids that spawn following lunar periodicity (Nemeth, 2005). Additionally, the presence of males within defended territories throughout the entire diver survey period contradicts migration patterns of lunar-based spawners (Nemeth *et al.*, 2007; Biggs & Nemeth, 2016) and cost-benefits of allocating energy to territorial defense without a reproductive return (Warner & Hoffman, 1980a). Extended spawning seasons that are uncoupled from lunar timing have been observed for *M. rosacea* within the Gulf of California (Erisman *et al.*, 2007) and additional species in the eastern Pacific (Adreani *et al.*, 2004; Adreani & Allen, 2008). If *M. jordani* follows similar trends, spawning may be possible throughout the entire season and be driven by local temperatures, seasonal upwelling, and associated increases in productivity (Adreani *et al.*, 2004; Kahru *et al.*, 2004; Erisman *et al.*, 2007; Adreani & Allen, 2008); however, additional surveys are required to confirm whether spawning occurred outside of our observations.

Sounds were recorded throughout all hours of the day, further indicating that sounds are not solely produced during territorial patrols, courtship, and spawning rushes. However, diel trends revealed an increase in the hours before spawning and closely followed observed patterns of courtship and spawning. While not surprising, this finding exemplifies that levels of recorded sounds can be utilized to generalize diel patterns of courtship and spawning activity when validated with observations (Schärer *et al.*, 2012a, 2014). Additionally, as sounds hr^{-1} changed as a function of time and behavioural activity, the sound production rates of individual males were not stationary, which is important to consider when interpreting data and estimating future relationships between long-term acoustic measurements and fish abundance (Rowell *et al.*, 2012, 2017).

IMPLICATIONS FOR MANAGEMENT

The results of this study increase our understanding of the mating system of *M. jordani* and help explain in part why the stock collapsed following extensive fishing effort at reported spawning aggregations from which an estimated 60 tonnes were harvested per month in May and June 1962 (Sáenz-Arroyo *et al.*, 2005). Territorial behaviours and protracted, seasonal site fidelity at spawning sites used across multiple years equates to a life strategy that is particularly vulnerable to overfishing as reproductive stocks can be easily located and removed over extended yet predictable periods of time (Sadovy de Mitcheson *et al.*, 2013). However, spatial and temporal fidelity also offer an opportunity to develop seasonal or areal protections for spawning aggregations of *M. jordani* that have proven successful in restoring other vulnerable species (Nemeth, 2005). Identifying and protecting spawning aggregations provides a measureable means to sustainably and cost-effectively manage fish population and restore ecosystem functioning and fisheries resources (Sadovy de Mitcheson, 2016; Erisman *et al.*, 2017). Our findings suggest that spawning likely occurs from March to June with additional months of species presence at aggregation sites. As the site described in this study is the only confirmed location of spawning, a conservative seasonal closure may assist in rebuilding the declining population until additional spawning sites are located and assessed for temporal patterns of reproductive activity. With the identification of additional spawning sites, combined areal and seasonal fisheries closures that target *M. jordani* may be able to replicate the population rebound successes witnessed in CPNP (Nemeth *et al.*, 2006; Aburto-Oropeza *et al.*, 2011).

Documentation of species-specific sounds produced by *M. jordani* within the behavioural contexts of courtship and spawning provides a basis for locating additional spawning sites throughout the species range and monitoring long-term patterns of sound production to deduce spawning activity across multiple sites (Rowell *et al.*, 2015). Using local knowledge or grey literature (Sáenz-Arroyo *et al.*, 2005), future efforts can be made to target potential spawning aggregation sites for confirmation using passive acoustic and visual survey methods. If differences in temporal patterns of sounds are found among sites, conservative protections, if implemented, can be adapted to account for regional idiosyncrasies. With an understanding of patterns and rates of behaviours, future acoustic modeling of the detection ranges of *M. jordani* sounds may yield a means to not only monitor acoustic and spawning activity but also a method to monitor changes in spawning stock abundance across the species' extensive geographic range. Future long-term monitoring of acoustic behaviours across multiple sites will enhance our current understanding of reproduction in *M. jordani* and support the development and assessment of pending management strategies.

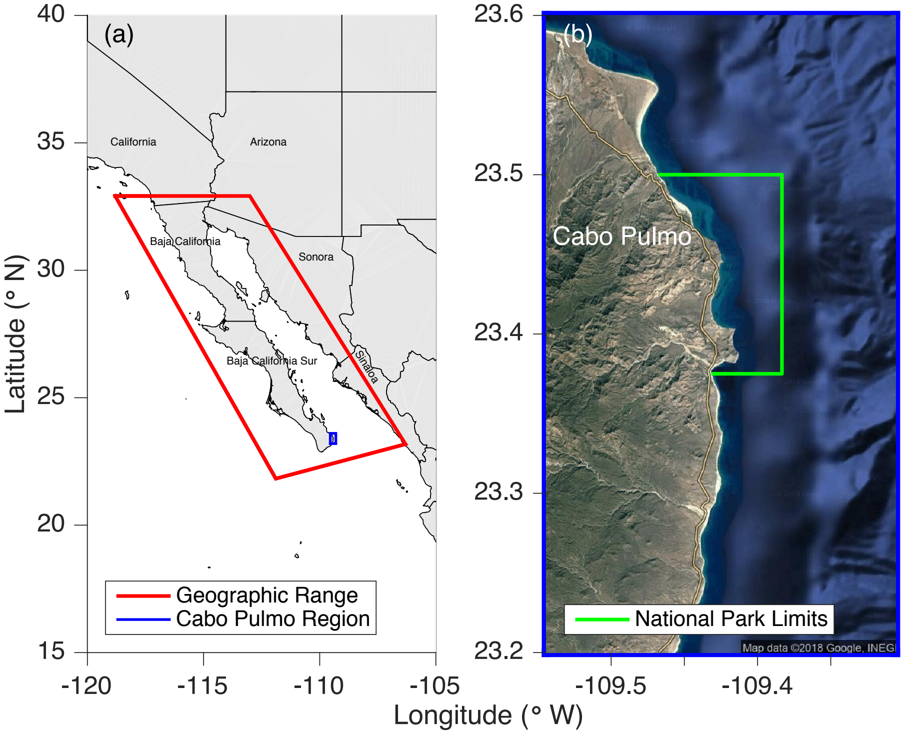


Figure 1.1. (a) The geographic range of *Mycteroperca jordani* in the Gulf of California and Pacific ocean stretches from La Jolla, California, USA to Mazatlán, Sinaloa, México. (b) Near the southern tip of the Baja California Peninsula (blue box in (a)), Cabo Pulmo National Park protects 71 km^2 of reef and sand habitats and a modest abundance of *M. jordani*. The location of the study site is omitted intentionally to mitigate potential poaching.

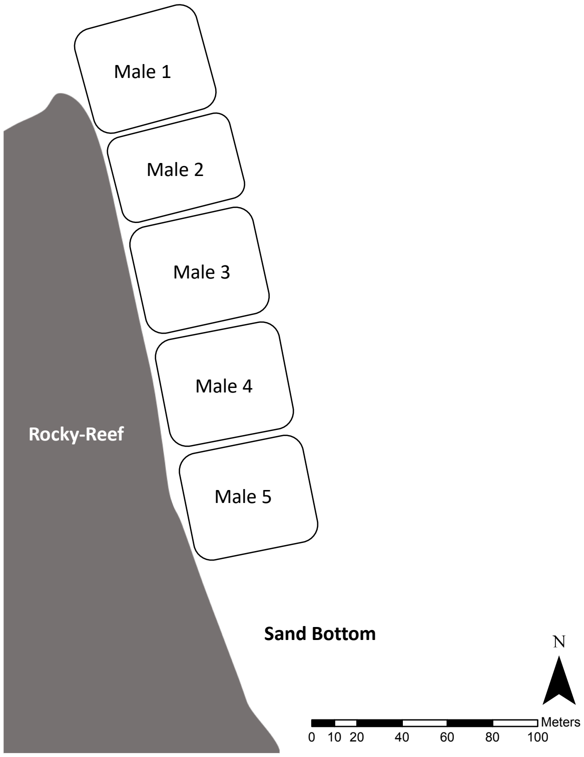


Figure 1.2. Territory locations of five males of *Mycteroperca jordani* present at specific locations within the survey area in May 2017. Territories extended over sand habitats seaward of a rocky reef and did not overlap spatially within observations. The shapes of territories were generalized based on GPS locations of estimated boundaries along the reef interface and spatially referenced observations of territorial patrolling behaviours seaward of the reef.

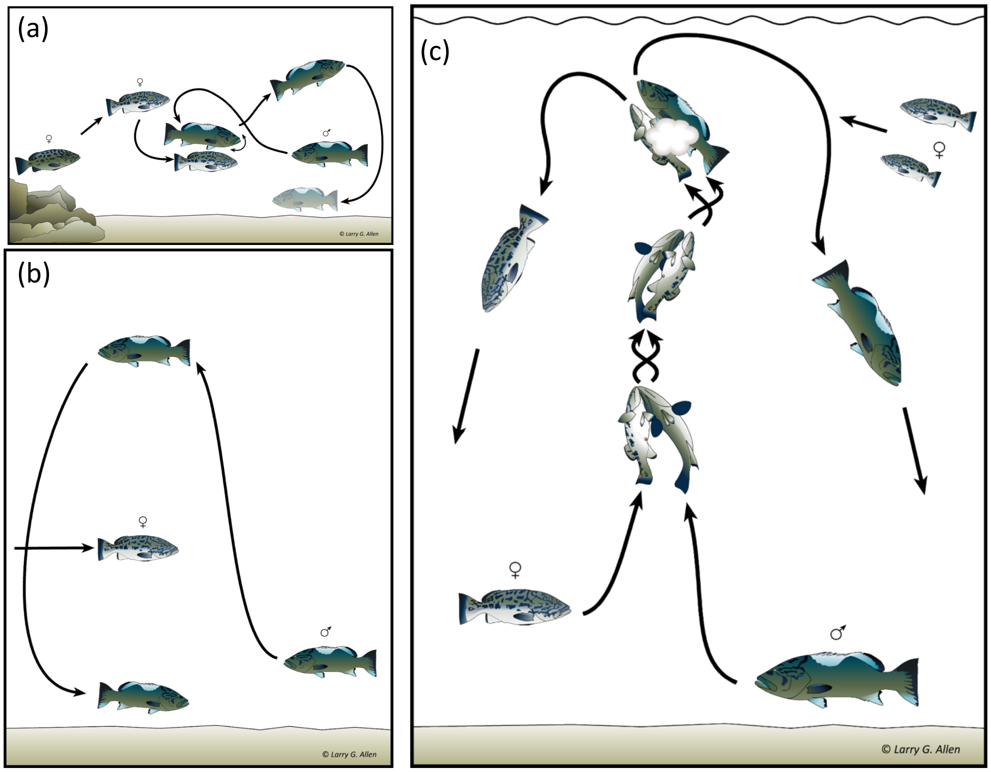


Figure 1.3. Illustrations of the courtship and pair spawning behaviour of *Mycteroperca jordani*. (a) Head shake: when located, a male courts a female through encirclement, head shakes, and a slight vertical rise. (b) Burst rise: more commonly observed, a male rapidly rises over a single female in a broad arching path before returning to the bottom. Both head shake and burst rise behaviours precede spawning. (c) Spawning rush: a male rises off the bottom at an angle and is joined by a single female rising at an opposing angle. The pair spirals upwards to approximately 2 m below the surface and gametes are released in upward thrusts. After gamete release, the male and female either return to the bottom separately or with the male chasing the female to the bottom. On occasion, additional females approach the pair after gamete release but do not enter the gamete cloud or release eggs. All behaviours typically include sounds produced by the male. (Image credit: Larry G. Allen).

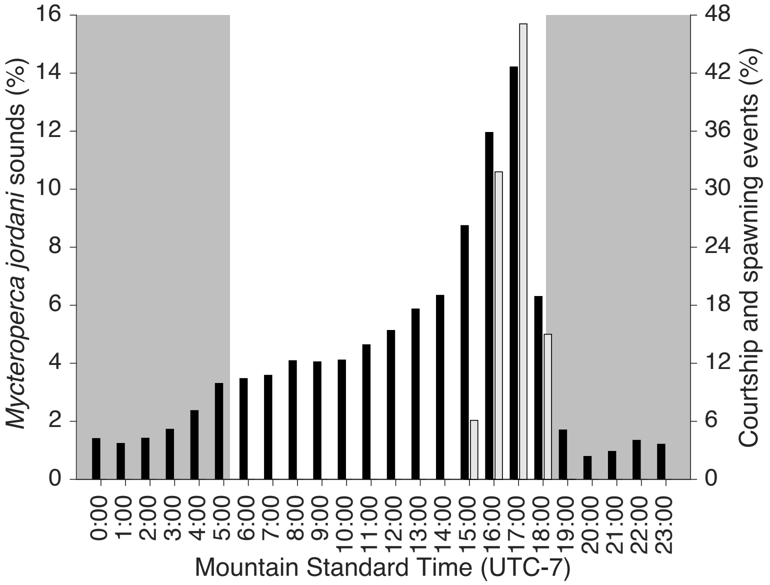


Figure 1.4. The percentage of *Mycteroperca jordani* sounds recorded in hour bins during long-term monitoring show a diel pattern of sound production (black bars). The percentage of courtship and spawning events (white bars) observed during diver surveys reveal similar trends prior to sunset, but surveys were only made from 1536 – 1911 h Mountain Standard Time. Proportions of events were weighted for observation time. Gray regions portray hours prior to sunrise and after sunset based on mean times for the monitoring period.

Figure 1.5. Colour phases of adult *Mycteroperca jordani*: (a, b) mottled phase of males and females not associated with reproductive behaviours, (c, d) camouflage phase of males and females when resting on the seafloor, (e, f) dark phase of males and females when swimming or stationary over reef habitat, (g) gray-head phase of males when not patrolling territories, courting females, or spawning, (h) silver phase of females when over sandy habitats and not interacting with males, (i) white-back phase of males when patrolling territories, courting females, and spawning, and (j) white-belly phase of females when present in male territories in evening hours and engaged in courtship and spawning behaviours with males. White-back and white-belly phases are associated with reproductive behaviours in males and females, respectively.

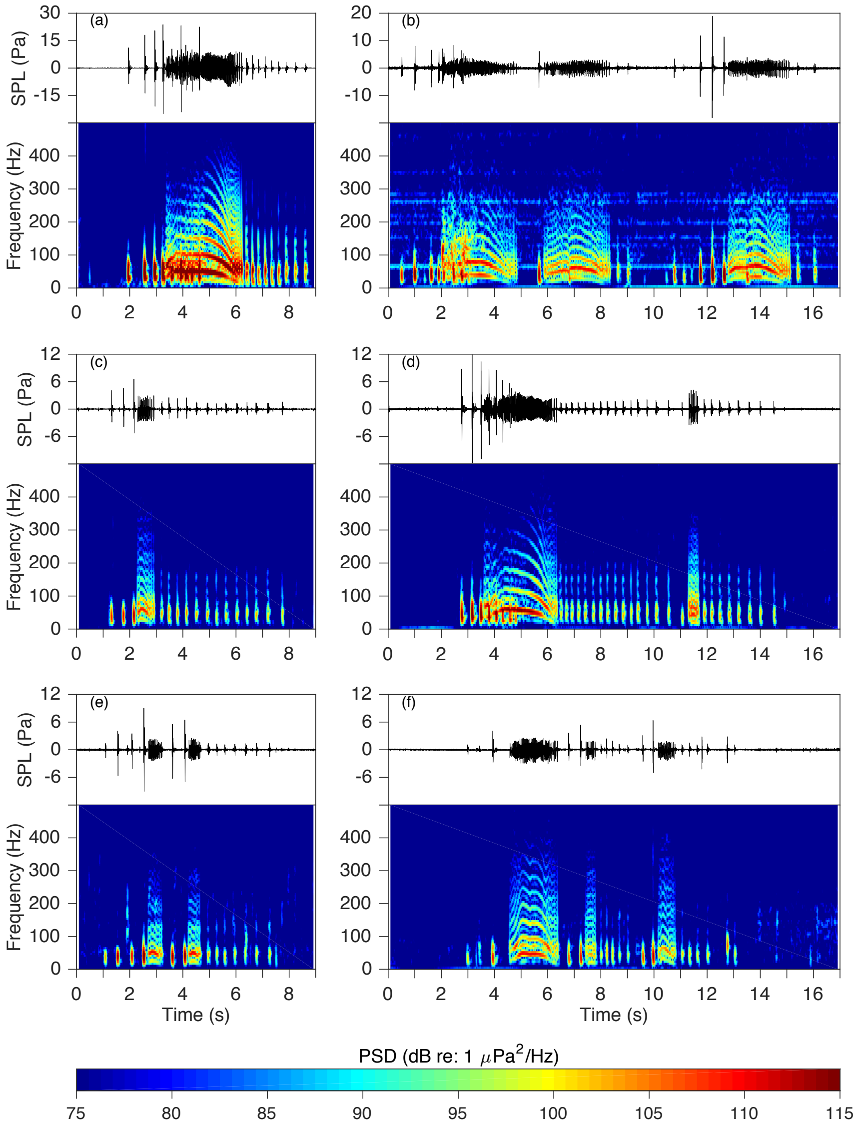


Figure 1.6. Oscillograms (top panels) and spectrograms (bottom panels) of (a) long and (c) short types of sounds as well as (b, d – f) multiple combinations confirmed to be produced by *Mycteroperca jordani* during patrolling, courtship, and spawning behaviours. SPL = sound pressure level measured as Pascals; PSD = pressure spectral density level (dB re 1 μPa^2 Hz^{-1}).

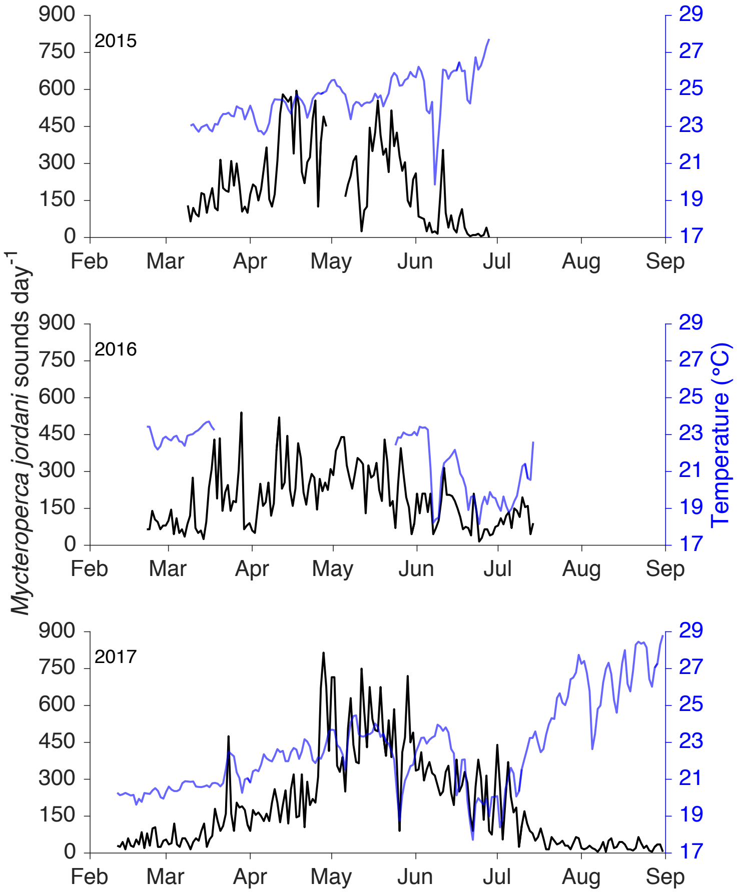


Figure 1.7. Time series of total *Mycteroperca jordani* sounds detected day^{-1} (black lines) and temperature (blue lines) during long-term monitoring in 2015, 2016, and 2017.

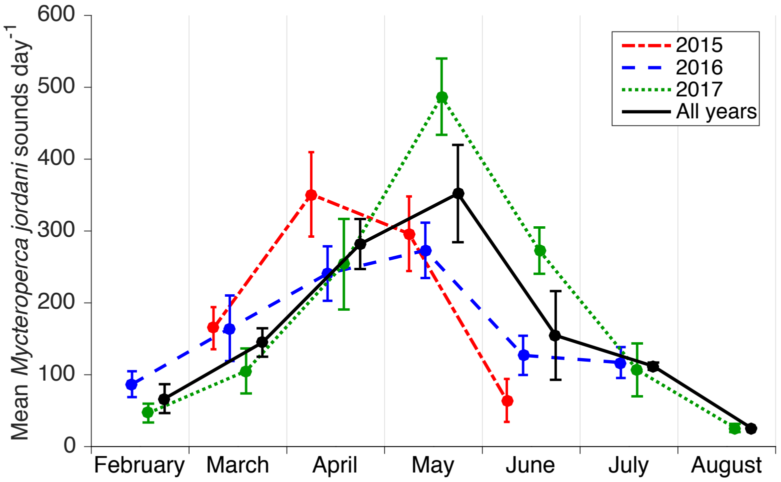


Figure 1.8. The mean number of *Mycteroperca jordani* sounds day^{-1} (± 95% C.I.) recorded during months of long-term acoustic monitoring in 2015 – 2017, and the monthly mean number of *M. jordani* sounds day^{-1} (± S.E.) across the three-year period of acoustic monitoring.

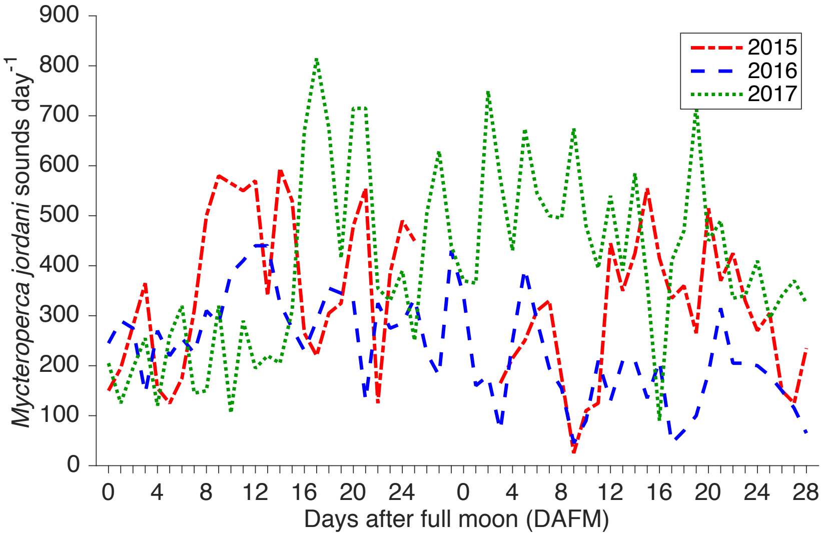


Figure 1.9. The total number of *Mycteroperca jordani* sounds recorded day^{-1} following the full moons in the months of April and May in 2015 – 2017. DAFM = days after full moon; full moon = 0 DAFM.

Table 1.1. Occurrences and descriptions of observed behaviours in adult *Mycteroperca jordani* during spawning periods in evening hours.

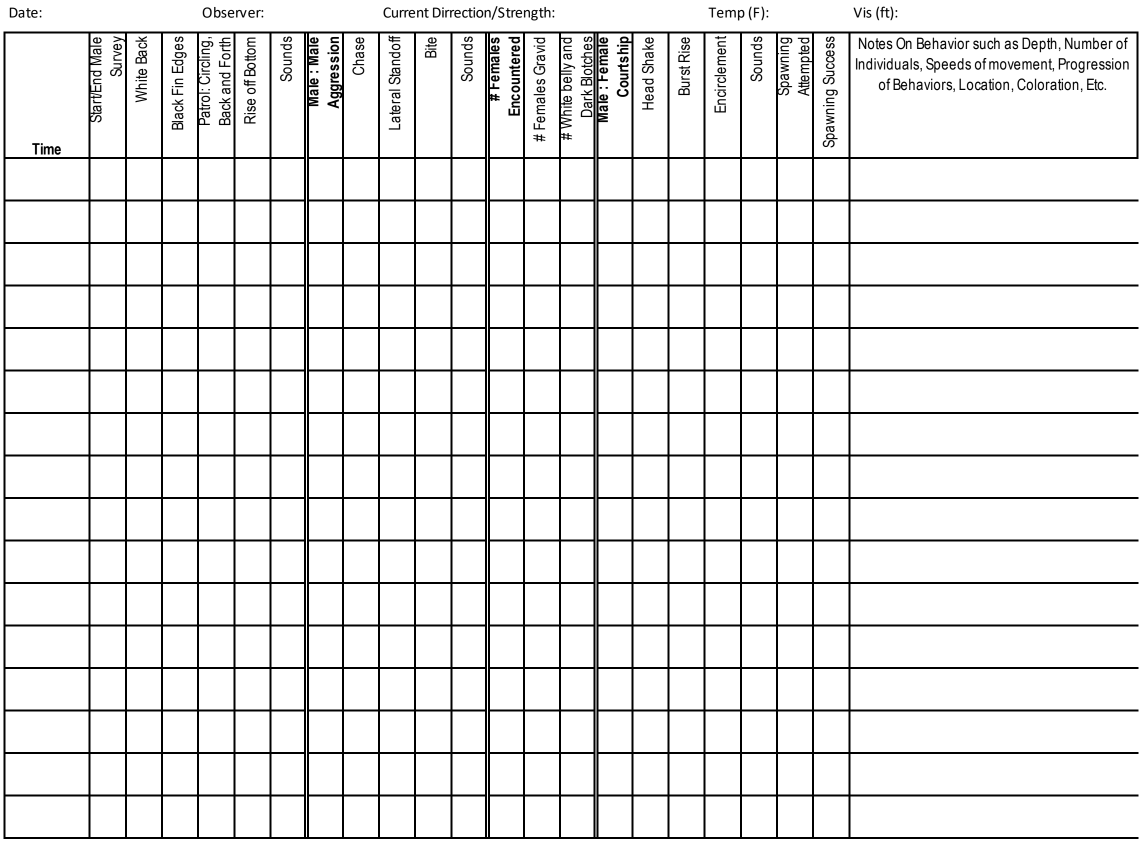
Behaviour	Occurrence	Description
Patrol	Territorial	A male swims rapidly 1 – 2 m off the bottom throughout his territory over sand. He swims along the boundary of his territory as well as the interior. Paths of patrols include swims from the reef interface to the seaward limits of territories over sand. Males unwaveringly patrol territories during evening hours.
Lateral standoff	Territorial	Two males meet at the boundary between their territories and swim slowly side by side. Males orient their bodies to laterally display the dorsal regions of their bodies. After interactions males return to their original territories.
Chase	Territorial	When encountering another male within his territory, the male rapidly chases the intruding male out of his territory and returns to patrolling. Chases of additional species out of territories can occur, *e.g.* bull sharks.
Head shake	Courtship	A single male encircles a single female, shakes the anterior portion of his body and head within 1 m of the female, and then moves away in a slight arching rise over her. This behaviour can occur on the bottom or in the mid-water column and precedes burst rises and spawning rush. Sounds are produced concurrent with head shake.
Burst rise	Courtship	A single male swims from the bottom and rises over a single female in an overarching path and returns to the bottom. The female is suspended in the water column during the burst rise and does not swim to unite with the male. Sounds are produced during burst rises.
Spawning rush	Spawning	A male rises off the bottom and swims in an upward angle towards a single female. The female rises at an opposing angle and joins the male. The pair adjoin their heads and spiral upward. Gametes are released in an upward thrust 2 m below the surface. The pair return to the bottom either separately or with the male chasing the female downward. Spawning rushes occur during evening hours. Sounds are produced during spawning rushes prior to gamete release.

Table 1.2. Characterization statistics of long and short sounds produced by *Mycteroperca jordani* during patrolling, courtship, and spawning behaviours. 95% C.I. = 95% confidence interval.

Sound/Part	Parameter	Mean ± 95% C.I.	n	Min	Max
Long Sound					
Pre-Tonal Pulses					
	Pulses sound^{-1}	3.9 ± 0.2	162	1	9
	Pulse duration (ms)	117.7 ± 2.2	638	54.8	225.2
	Interpulse duration (ms)	278.8 ± 9.2	476	45.7	674.1
	Fundamental frequency (Hz)	38.8 ± 0.8	638	17	63
	3 dB bandwidth (Hz)	13.2 ± 0.5	638	4	44
	6 dB bandwidth (Hz)	21.0 ± 0.8	638	6	55
Tonal Part					
	Duration (ms)	2394.5 ± 79.1	162	1177.8	4067.9
	Fundamental frequency (Hz)	49.8 ± 1.0	162	34	79
	3 dB bandwidth (Hz)	2.8 ± 0.2	162	1	10
	6 dB bandwidth (Hz)	4.9 ± 0.4	162	2	19
Post-Tonal Pulses					
	Pulses sound^{-1}	7.5 ± 0.6	162	0	18
	Pulse duration (ms)	103.5 ± 1.4	1219	48.2	229.7
	Interpulse duration (ms)	240.2 ± 7.8	1066	59.6	1156.0
	Fundamental frequency (Hz)	46.3 ± 0.6	1219	12	83
	3 dB bandwidth (Hz)	14.5 ± 0.3	1219	5	64
	6 dB bandwidth (Hz)	21.9 ± 0.5	1219	7	84
Short Sound					
Pre-Tonal Pulses					
	Pulses sound^{-1}	2.4 ± 0.4	42	0	6
	Pulse duration (ms)	92.9 ± 4.0	99	47.6	137.5
	Interpulse duration (ms)	357.6 ± 28.3	58	65.1	798.0
	Fundamental frequency (Hz)	39.1 ± 2.2	99	20	61
	3 dB bandwidth (Hz)	16.6 ± 1.4	99	6	43
	6 dB bandwidth (Hz)	27.2 ± 2.3	99	9	53
Tonal Part					
	Duration (ms)	666.5 ± 70.4	42	295.0	1144.8
	Fundamental frequency (Hz)	52.3 ± 2.8	42	33	84
	3 dB bandwidth (Hz)	3.2 ± 0.4	42	1	7
	6 dB bandwidth (Hz)	6.6 ± 1.3	42	2	23
Post-Tonal Pulses					
	Pulses sound^{-1}	4.2 ± 1.1	42	0	13
	Pulse duration (ms)	106.7 ± 2.5	177	53.0	155.5
	Interpulse duration (ms)	251.1 ± 20.1	147	60.0	903.4
	Fundamental frequency (Hz)	45.4 ± 1.8	177	17	89
	3 dB bandwidth (Hz)	14.1 ± 0.7	177	7	40
	6 dB bandwidth (Hz)	20.8 ± 1.0	177	10	52

APPENDICES

Supporting Information Table S1. Data collection sheet used in the study to record behavioral observations of *Mycteroperca jordani* at a spawning aggregation.



Date: Observer: Current Dirrection/Strength: Temp (F): Vis (ft):

Time	Start/End Male Survey	White Back	Black Fin Edges	Patrol: Circling, Back and Forth	Rise off Bottom	Sounds	**Male : Male Aggression**	Chase	Lateral Standoff	Bite	Sounds	**# Females Encountered**	# Females Gravid	# White belly and Dark Blotches	**Male : Female Courtship**	Head Shake	Burst Rise	Encirclement	Sounds	Spawning Attempted	Spawning Success	Notes On Behavior such as Depth, Number of Individuals, Speeds of movement, Progression of Behaviors, Location, Coloration, Etc.

ACKNOWLEDGEMENTS

The authors would like to thank D. Castro-Arvizu, C. Biggs, B. Martinez-Guerrero, T. TinHan, A. Johnson, A. Ramirez-Valdez, M. Doane, E. Reed, and staff of Cabo Pulmo Divers for field assistance, members of the Centro Para la Biodiversidad Marina y Conservación A.C., Comisón Nacional de Áreas Naturales Protegidas (CONANP), and Parque Nacional Cabo Pulmo for logistical and permitting support, and G. L. D'Spain, P. A. Hastings, B. X. Semmens, J. B. Shurin, and D. A. Demer for intellectual guidance. We thank L. G. Allen for creating ethograms for this study. The project was funded in part by grants to O.A. and B.E.E. from the Walton Family Foundation, the David and Lucile Packard Foundation, and the World Wildlife Fund México.

Chapter 1, in full, was submitted for publication as: Rowell, Timothy J.; Aburto-Oropeza, Octavio; Cota-Nieto, Juan José; Steele, Mark A.; Erisman, Brad E. (*in review*). Reproductive behaviour and concurrent sound production of Gulf grouper *Mycteroperca jordani* (Epinephelidae) at a spawning aggregation site. *Journal of Fish Biology*. The dissertation author is the primary investigator and author of this manuscript.

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CHAPTER 2

Drivers of male sound production and effective communication ranges at fish spawning aggregation sites

ABSTRACT

The monitoring of fish sounds during reproductive periods has enhanced our understanding of spatio-temporal patterns of spawning activity and potential impacts of ambient noise on fish communication. The interpretation of measures of fish sounds obtained from recordings of the received acoustic field often fails to incorporate the environmental effects of ambient noise levels (NL) and transmission loss (TL), and signal characteristics on the probability of detecting fish sounds within different periods of time, resulting in uncertainty of whether these measures of detected fish sounds correspond to acoustic activity and associated behaviors of the fish themselves or are caused by the environment in which the recordings are made. In this study, we applied acoustic propagation modeling and detection theory to estimate rates of sound production for territorial male Gulf grouper (Mycteroperca jordani) from acoustic recordings and evaluate effective communication ranges during reproductive periods. To assess potential drivers of sound production, environmentally-calibrated estimates of acoustic activity in each hour of monitoring were compared to observations of rates of courtship, spawning, and females encountered within male territories. During the study, the acoustic recorder effectively sampled the sounds of between 2 – 5 males depending on varying NL within each hour. Rates of sound production showed diel patterns and daily variation that were indicative of observed rates of spawning rushes and females encountered within male territories. The effective communication range was estimated to be

less than 21 m, which confirmed the importance of short range communication within the observed behaviors and highlighted potential impacts of ambient noise on communication. Our findings corroborate that measures obtained from passively recorded fish sounds can be used to infer reproductive activity and relative abundance if properly calibrated for environmental effects and detection capabilities.

INTRODUCTION

Continued efforts to understand the sound producing capabilities of fishes have increasingly identified the importance of acoustic communication in behaviors associated with distress, aggression, and reproduction for over 100 families of fishes (Amorim, 2006; Webb *et al*., 2008a; Lobel *et al.*, 2010; Ladich, 2015), resulting in the increased monitoring of fish sounds to better understand assemblages, populations, and behaviors (Rountree *et al.*, 2006; Luczkovich *et al*., 2008). Sound production associated with courtship and reproductive displays is perhaps the most well-studied area of fish bioacoustics, with a diverse assemblage of fish families known to produce sounds during or prior to spawning (Myrberg & Lugli, 2006; Lobel *et al.*, 2010; Schärer *et al*., 2014; Bertucci *et al.,* 2015). Sounds associated with reproductive behaviors can function to attract mates to male territories (Brantley & Bass, 1994), express reproductive fitness (Rowe & Hutchings, 2004; Vasconcelos *et al.*, 2012), and coordinate synchronized migrations to, and spawning at, aggregation sites (Rowell *et al.*, 2015; Erisman & Rowell, 2017). With an understanding of the behavioral contexts of sounds produced during reproductive periods, acoustic monitoring can be effective in documenting spatio-temporal patterns of spawning and possibly fish abundance (Rowe & Hutchings, 2006; Mann *et al.*, 2010; Rowell *et al.*, 2012; Rice et *al.*, 2017; Rowell *et al*., 2017).

The interpretation of acoustic data recorded with a single hydrophone to deduce patterns of reproductive activity and fish abundance often relies on either detecting and enumerating fish sounds or estimating contributions of fish sounds to ambient noise through measures of sound pressure levels over discrete durations (Locascio & Burton, 2016; Monczak *et al*., 2017). While valuable in documenting levels of fish sounds produced during reproductive periods, these inferential approaches are limited by the uncertainty of whether different measures are indicative of differences in the number of sound producers (e.g., abundance), differences in rates of sound production produced by a fixed number of sound producers (e.g., sounds per individual per unit time), differences in the detectability of sounds as a function of time, or a combination of all factors (Zimmer *et al*., 2008; Helble *et al.*, 2013a; Küsel *et al*., 2011; Marques *et al*., 2013). The importance of understanding these uncertainties is particularly evident for species in which only males produce sounds from within established reproductive territories (Mann *et al*., 2010; Tricas & Boyle, 2014). Assuming ambient noise levels are not varying significantly with time, temporal variations in detected sound levels generated by territorial species are not necessarily attributable to changes in male abundance but rather likely reflect changes in rates of sound production that may be function of hour, day, and reproductive activity (Amorim *et al*., 2003; Montie *et al*., 2016, 2017). However, as there is evidence that rates of sound production by territorial males is in part influenced by the presence of females (Bremner *et al*., 2002), changes in sound levels resulting from varying rates of sound production may be indicative of both reproductive activity and the abundance of both sexes.

The ability to estimate rates of sound production by individuals and calibrate levels of sounds detected to account for environmental effects facilitates a more robust interpretation of acoustic recordings, permitting examinations of influential drivers of observed sound levels (Johnson *et al*., 2018). Such an undertaking requires knowledge of the numbers and distributions of sound producers within a study site as well as an understanding of acoustic propagation and the probability of detecting a sound in variable ambient noise conditions (Küsel *et al*., 2011; Helble *et al*., 2013a). While challenging for species that exhibit transient distributions, numbers and distributions of sound producers can be estimated for territorial species through visual observations or multi-hydrophone arrays (Shapiro *et al*., 1993; Gladstone, 1994; Parsons *et al*., 2009; Locascio & Mann, 2011b). The probability of detecting fish sounds as a function of range, azimuth, depth, and ambient noise levels (NL) can be estimated through acoustic propagation models of transmission loss (TL) and an understanding of source levels (SL) and detection thresholds (DT; Zimmer *et al*., 2008; Helble *et al*., 2013b). By coupling knowledge of fish distributions and estimates of maximum detection ranges, measures of sounds detected can be attributed to the number of sound sources within the detection range of an acoustic recorder in a given period of time (Marques *et al*., 2013; Buckland *et al*., 2015), permitting estimates of rates of sound production per individual. These calibrated estimates of acoustic activity can be compared to data of potential drivers, such as behavioral observations, spawning activity, and female abundance (Johnson *et al*., 2018).

The inclusion of acoustic propagation modeling into studies aimed at interpreting passive acoustic recordings of fish sounds to deduce reproductivity activity and abundance also provides an opportunity assess the hearing capabilities of target species within the environment (Stanley *et al*., 2017). Similar to estimating detection ranges of an acoustic recorder for a given source under given environmental conditions, the hearing ranges of sounds produced and received by conspecifics can be estimated under different ambient noise conditions by determining the ranges at which reductions in received sound levels (RL) from TL fall below the hearing threshold of fishes or are masked by ambient noise (Tavolga, 1974; Clark *et al*., 2009; Codarin *et al*., 2009; Slabbekoorn *et al*., 2010). Foundational information about the effective communication ranges of fishes is not only important for understanding the relevance of acoustic communication over short and long distances within reproductive contexts but also provides an initial assessment of how communication may be affected by anthropogenic noise in the oceans (Slabbekoorn *et al*., 2010; Radford *et al*., 2014; Stanley *et al*., 2017). Acoustic signaling by males likely conveys information about fitness to prospective mates and contributes to reproductive success (Rowe & Hutchings, 2004; Lobel *et al*., 2010; Vasconcelos *et al*., 2012; Amorim *et al*., 2015). Thus, baseline measurements of effective communication ranges are needed to anticipate and mitigate potential impacts of man-made sound on the reproductive success of species (Slabbekoorn *et al*., 2010; Radford *et al*., 2014).

In this study, we sought to: (1) estimate the sound production rates of individual fish as a function of time using acoustic propagation modeling and observations of fish distributions at a spawning aggregation site, (2) evaluate whether levels of sound production rates are indicative of levels of reproductive activity and relative abundance, and (3) estimate the effective communication range of conspecifics to assess the importance and role of acoustic communication during reproductive behaviors, using Gulf grouper (*Mycteroperca jordani*) as a case study. Gulf grouper was selected as an appropriate species to address these objectives as the acoustic behaviors and mating system of Gulf grouper are now understood (Rowell *et al*., *in review*). These behaviors include the establishment of fixed male territories at low fish densities (e.g., Rowell *et al*., *in review*). We anticipate that the results of this study will provide a foundation for similar studies of other species with similar reproductive attributes.

MATERIALS AND METHODS

STUDY SITE AND SPECIES

Passive acoustic and observational monitoring of the protogynous Gulf grouper (*Mycteroperca jordani*) was conducted at a spawning aggregation site of the species within the protective boundaries of Cabo Pulmo National Park (CPNP), Baja California Sur, México. Since its establishment in 1995, CPNP has supported the recovery of Gulf grouper and other large top predators (Aburto-Oropeza *et al*., 2011). Spawning at an aggregation site in CPNP has been observed over the 3-year period 2015 – 2017. The aggregation site is located in a habitat with approximately constant 18 m of water over a sandy bottom. From March to June, male Gulf grouper establish discrete territories over sand that are patrolled, defended, and used as courtship and spawning arenas (Rowell *et al*., *in review*). As part of spawning bouts, males court and pair-spawn with individual females that arrive to their territories during the hours prior to sunset (Rowell *et al*., *in review*). Males produce sounds in close proximity to females (less than 10 m range) during courtship and spawning rushes. These sounds have a mean fundamental frequency of 50 Hz and additional harmonics at approximately 100, 150, 200, 250 Hz, and upward (Figure 2.1). Sound production by females has not been observed. Gulf grouper is currently listed as an endangered species by the International Union for the Conservation of Nature and Natural Resources (IUCN) due to a decreasing trend in population size (Craig *et al*., 2008). It currently is estimated to be 1% of historical levels that were present prior to extensive fishing pressure and the collapse of the stock in the 1970s (Rámirez-Rodriguez *et al*., 1996; Saenz-Arroyo *et al*., 2005). Until recently, little was known about the reproductive behaviors of the species (Rowell *et al*., *in review*). Thus, efforts to understand the drivers of sound production rates and capabilities of acoustic communication by Gulf grouper will enhance our understanding of the acoustic behaviors of fishes while providing warranted information about an endangered, data-limited species.

ACOUSTIC MONITORING AND ANALYSIS OF SOUNDS

From 6 May to 23 May 2017, underwater sound was recorded at a fixed location between the territories of two males over a sandy-bottom habitat with a SoundTrap 300 STD single hydrophone, underwater acoustic recorder. This acoustic recorder, made by Ocean Instruments NZ, provides large dynamic range (16-bit A/D converter), broadband (20 Hz up to 60 kHz) recordings. Its full scale rms pressure response at 250 Hz is 171.9 dB re 1 μPa, equivalent to a total system sensitivity of -84.6 dB re 1 count μPa^{-1}. The recorder was attached to a stainless steel rod and concrete base with the hydrophone 1 m above the seafloor at 17 m depth. Underwater sound was recorded at a duty cycle of 1 min every 5 min and digitized at a sample rate of 48 kHz. Calibrated spectrograms were generated for each 1-min file (FFT length = 16384 points, Kaiser-Bessel window with beta = 7.85, FFT overlap of 75%) and analyzed manually. The number of Gulf grouper sounds per file with at least the first two harmonics (~50 Hz and ~100 Hz; Figure 2.1) that were detectable visually was recorded, then summed over consecutive, non-overlapping 1-hr bins, and finally multiplied by five to account for duty cycle sampling to estimate the total number of sounds detected per hr.

Files containing Gulf grouper sounds with high signal-to-noise ratios that were produced at known distances from the hydrophone as estimated by divers (see below) were selected to estimate the dominant frequencies of the first three harmonics of the sounds, their source levels, and their detection thresholds for a human analyst. Pressure spectral density (PSD) levels in units of dB re 1 $\mu Pa^2 Hz^{-1}$ were calculated (FFT length = 48000 points, Hanning window with length = 48000, FFT overlap of 0%), and the maximum PSD levels within adjacent, non-overlapping 50 Hz-wide bands were identified to estimate the dominant frequencies of the first three harmonics (Figure 2.1). TL (dB) for each sound was estimated as loss resulting from spherical spreading over the estimated source range (r; m) from the hydrophone (i.e., $TL = 20\ log_{10}(r/1\ m)$). TL was added to received levels after integrating the PSD over 1-Hz bands centered on the maximum PSD levels to estimate the SL (dB re 1 μPa at 1 m) of each harmonic frequency. The DT of the analyst that recorded occurrences of Gulf grouper sounds within spectrograms was assessed by determining the minimum signal-to-noise ratio required for each harmonic to be visually detectable within spectrograms. Increasing levels of Gaussian white noise were added to the pressure time series in each file, and spectrograms were created and viewed with the same settings and computer screen used to detect Gulf grouper sounds. Upon reaching an input level of Gaussian white noise when a harmonic frequency was no longer detectable by the analyst, PSD levels were calculated for the background noise and Gulf grouper signal. For each dominant harmonic frequency, PSD levels of background noise were subtracted from PSD levels of the signal to estimate the analyst DT (dB) for each of the first three harmonic frequencies. Mean dominant frequencies, SL, and analyst DT for each harmonic were calculated in the linear domain and converted to dB when appropriate.

NL (dB re 1 μPa) at the mean dominant frequencies of the first three harmonics was estimated for each 1 min recording from the PSD levels (after integrating over the 1-Hz band, as before). Mean NL for each hour of recording was calculated for the three frequencies. Time series of mean NL per hr were generated to examined changing levels of background noise throughout the study period and assess potential impacts on the detection of Gulf grouper sounds in recordings. The mean, minimum, and maximum NL per hr were calculated for the entire study period.

OBSERVATIONAL MONITORING

From 6 – 10 and 12 – 23 May 2017 (17 days), observational monitoring of the spawning aggregation site was conducted by divers to study the mating system and behaviors of Gulf grouper (e.g., Rowell *et al.*, *in review*). During morning (0800 – 1100 Mountain Standard Time; MST; UTC – 7 hours) and evening hours (1800 – 1900 MST), divers conducted video-transect surveys across the site to estimate the spatial distribution and boundaries of territories for individual males that were identified by unique body markings, caudal fin shape, and scarring patterns. After observing individual males within fixed territories during morning and evening surveys, divers released surface marker buoys at the estimated centers and boundaries of male territories. The locations of the buoys were recorded with a handheld GPS (GPSMap 78; Garmin) by the captain of the dive vessel. Locations were mapped and used to estimate the ranges of territorial boundaries from the fixed acoustic recorder.

Additional evening surveys (1600 – 1800 MST) were conducted by a diver team on each of the 17 days to document the reproductive behaviors of a single, focal male whose territory abutted the location of the fixed acoustic recorder. During each survey, divers followed the male and recorded observations of behaviors associated with spawning bouts and females present within the male territory. The time of observations and duration of surveys also were recorded. All data were verified from video recorded with GoPro cameras carried by divers. Recorded behaviors included:

head shakes: a male approaches a single female and shakes the anterior portion of his body and head, *burst rises*: a male swims from the seafloor to the mid-water column in an arching path over a single female, and

spawning rushes: a male and female pair unite in the water column, swim upward, and release gametes.

Males produce sounds at depths of 5 – 18 m during all behaviors, but with the exception of spawning rushes, the inclusion of sounds within each behavior has not been observed to be obligatory (Rowell *et al.*, *in review*). While following the male, divers documented the number of females separately encountered within the territory. The number of behavioral observations and females encountered were summed per hour for each day, divided by survey duration, and multiplied by 60 min to estimate the occurrence rates of each variable per hr in the 1600, 1700, and 1800 hours. As the duration of the surveys was less than 1 hr (mean: 24 min), we assumed that observed rates were representative of rates outside the period of observations during the 1600 – 1800 hours. Observations of sound production were also noted, and for sounds produced in close proximity to the acoustic recorder, the source distance from the receiver was estimated and used to estimate SL of the first three harmonics (see above).

ACOUSTIC PROPAGATION MODELING

To assess the detectability of Gulf grouper sounds by the acoustic recorder and conspecifics, a range-and-azimuth-independent geoacoustic model of the environment was developed and acoustic propagation modeling was performed at each harmonic frequency to estimate TL as a function of range, source depth, and receiver depth at the site. The geoacoustic model of the site was estimated from data collected *in situ* (water depth, sound speed profile, and sediment properties at the ocean-sea bottom interface) and empirical equations published in the scientific literature (profiles of density, compressional speed, and compressional attenuation in the sea bottom layers). The uppermost unconsolidated sediment layer was modeled as a fluid. The environmental properties consisted of a 18 m water column with uniform temperature and density, a 50 m thick coarse grain sediment layer, and a basalt bottom half-space. The full geoacoustic profile and sources of data and empirical equations are provided in Table 2.1. This environmental model was used as input to RAMGeo and Kraken acoustic propagation models available within the Ocean Acoustic Toolbox (Ocean Acoustic Library; oalib.hlsresearch.com). RAMGeo is based on the parabolic equation approximation to the acoustic wave equation, and Kraken solves the acoustic wave equation numerically using a normal mode decomposition. Sensitivity analyses were completed to determine the sensitivity of the output TL to changes in the input values of sediment thickness, compressional speed, and source depth. RAMGeo and Kraken then were run for the first three harmonic frequencies of Gulf grouper sounds (50 Hz, 100 Hz, and 150 Hz) using the best estimate of the input environmental model. The results of TL as a function of range and source depth to a receiver depth at 17 m for both acoustic codes with the same input geoacoustic model were compared. Due to the agreement of predicted TL among models (Figure 2.2), all further analyses were conducted with the Kraken normal mode propagation model so that the results could be more easily interpreted. Kraken was run for signal source depths of 5 – 18 m, generating estimates of TL as function of range and depth for the three frequencies.

RATES AND PREDICTORS OF SOUND PRODUCTION

To estimate the distances from the fixed acoustic recorder (at 17 m depth) in which Gulf grouper sounds could be detected in spectrograms by an analyst throughout the recording period, the modified sonar equation (Eq. 1) was used to estimate TL when signal excess (SE) equaled zero (Eq. 2) for each frequency, which approximates the 50% probability of signal detection (Clark *et al*., 2009).

$$SE = SL - TL - NL - DT \qquad \text{Eq. 1}$$

$$TL = SL - NL - DT \qquad \text{Eq. 2}$$

SL and analyst DT were fixed at the mean values for each of the three harmonic frequencies (see above), while NL varied for each hour of recordings as previously estimated. The study assumed that SL and DT did not vary with NL. TL at SE = 0 was calculated for each hour of recordings. For each hour, the maximum range from the acoustic recorder in which the resulting thresholds of TL were reached was estimated from TL values generated by Kraken for source depths of 5 – 18 m to a receiver depth of 17 m. The mean of these maximum distances of detection across source depths was calculated to estimate the mean range of detection of each harmonic frequency per hr, as the distribution of source depths was assumed to be random with a uniform probability distribution over the 5-18 m depth interval.

With an understanding of the spatial distribution of male territories and the mean detection range per hr, the number of males that were within the acoustic detection range of the recorder was estimated for each hr for each harmonic frequency. As the first two harmonic frequencies were required to be detectable by the analyst in order to be counted as a detection, the number of sounds detected per hr were divided by the number of males within the mean detection range of the second harmonic frequency to estimate the sound production rate per individual per hr. Resulting time series of estimated sound production rates per hr were created.

Sound production rates within the 1600 – 1800 hours were compared with hourly rates of behaviors and females encountered as estimated by divers to examine the importance of these variables on rates of acoustic activity. Correlations between sound production rates and rates of head shakes, burst rises, spawning rushes, and females encountered were tested with Spearman rank correlation tests ($\alpha = 0.05$), as the data did not meet the criteria of parametric testing. General linear models (GLM) were fitted to each pair of data. If predictor variables were correlated to sound production rates, qualified models were compared using Akaike's information criteria (AIC). To further rank the importance of predictor variables, a random forest model was fitted to the data using hour of day, head shakes per hr, burst rises per hr, spawning rushes per hr, and females encountered per hr as predictor variables and sound production rates per hr as the response variable (Breiman *et al*., 1984; Strobl *et al*., 2009). The number of regression trees used in the model was 200. The model was evaluated by estimating the variability around the mean from out-of-bag predictions. The importance of predictor variables in explaining sound production rates was ranked by permuting out-of-bag observations among decision trees.

EFFECTIVE COMMUNICATION RANGES

To estimate the effective communication ranges of Gulf grouper at the aggregation site, the modified sonar equation was used to estimate TL when SE = 0 (Eq. 2) for the first three harmonic frequencies using the DT of conspecifics obtained by reviewing available literature for DT of related species. SL was set as the mean SL for each harmonic. The closest phylogenic relative with a reported DT at the frequencies of Gulf grouper was *Sebastes schlegeli* (Family Scorpaenidae) with a DT of 27 dB (Motomatsu *et al*., 1998). Thus, the DT of Gulf grouper was estimated and fixed as 27 dB. TL at SE = 0 was calculated for the mean, minimum, and maximum NL per hr observed during the recording period for each frequency. If the solved TL was a negative number, a TL threshold at SE = 0 was considered equal to zero dB, and signal detection was therefore not possible. As the depths of both the source (i.e., males) and receiver (i.e., conspecifics) were observed to be variable within depths of 5 – 18 m during diver surveys, the maximum range in which a conspecific could detect a source signal as a function of different source and receiver depths was estimated from the TL values generated by Kraken for the three NL conditions. Estimates of the mean effective communication range for each NL condition were calculated by averaging maximum detection ranges over all source and receiver depths.

RESULTS

ACOUSTIC MONITORING AND ANALYSIS OF SOUNDS

The amount of Gulf grouper sounds detected per hr in the acoustic recordings was variable among days and hours but revealed diel increases in the hours prior to sunset (Figure 2.3). The ability of an analyst to detect the different harmonic components of the sounds and ultimately document the number of sounds per hr with the first two harmonics detectable was in part influenced by the SL and analyst DT of the first three harmonic frequencies, which were estimated from 15 files with Gulf grouper sounds that were produced at observed distances from the acoustic recorder. Harmonics above a center frequency of 150 Hz were rarely detected in the acoustic recordings and thus were not included in the analyses. The mean ± 95% C.I. dominant frequencies of the first three harmonics were 49.7 ± 1.9 Hz, 99.5 ±3.7 Hz, and 149.8 ± 5.1 Hz with mean ± 95% C.I. SL of 132.8 ± 3.1 dB, 115.7 ± 2.9 dB, and 107.3 ± 2.3 dB (re 1 μPa), respectively. The mean ± 95% C.I. analyst DT was estimated as 9.7 ± 1.1 dB, 7.6 ± 1.9 dB, and 8.8 ± 2.2 dB for the first three harmonics. Given the estimates, the first three harmonics were modeled as 50 Hz, 100 Hz, and 150 Hz signals.

Mean NL per hr at the site was variable as a function of time for the three frequencies (Figure 2.4). The mean NL per hr ranged from 73.5 dB to 99.7 dB dB re 1 μPa for 50 Hz (mean ± S.E. = 83.4 ± 0.2 dB), 71.8 dB to 97.1 dB for 100 Hz (mean ± S.E. = 79.5 ± 0.2 dB), and 69.6 dB to 91.9 dB for 150 Hz (mean ± S.E. = 76.5 ± 0.2 dB re 1 μPa). The large range of NL throughout the study period supported the assumption that temporal variability in NL impacted time series of the estimated number of sounds per hr (Figure 2.3) to a larger degree than the potential temporal variability of SL and DT in different ambient noise conditions, but this was not statistically confirmed with the data available. Increases in NL were observed during hours of morning and evening surveys at the site and were attributed to inputs in noise from boat operation and SCUBA diving.

OBSERVATIONAL MONITORING

During morning and evening surveys, divers confirmed the presence of five males within fixed territories at the study site. Individual males maintained the same territories throughout all hours and days of observations. The nearest territorial boundaries of the five males were estimated to be at ranges of 0 m, 1 m, 63 m, 115 m, and 160 m from the location of the acoustic recorder.

From 1600 to 1800 hours, divers observed and recorded variable rates of head shake, burst rise, and spawning rush behaviors and females encountered (Figure 2.5). No observations of any behaviors or female presence were made within the male territories on 6 May, but modest levels of behavioral activity and females present were documented on 7 – 10 May. From 12 – 23 May, higher rates of all behaviors and females encountered were observed and associated with increases in spawning activity. On average, rates of observations were highest in the 1700 hr followed by the 1600 hr and 1800 hr, respectively (Figure 2.5).

RATES AND PREDICTORS OF SOUND PRODUCTION

Kraken produced estimates of TL for 50 Hz, 100 Hz, and 150 Hz signals as a function of range and source depth to the acoustic recorder at 17 m that were used to estimate the detection ranges of Gulf grouper sounds by the analyst (Figure 2.6). On average, TL increased with range from the source across all frequencies, but increases in complexity of the TL curves were seen for 100 Hz and 150 Hz signals due to constructive/destructive interference of multiple normal modes. TL was reduced for sources at depths near the seafloor due to improved efficiency in mode excitation.

Calculations of TL at SE = 0 for each hour of recordings with varying NL per hr ranged from 23.3 dB to 49.6 dB for the 50 Hz harmonic (mean ± 95% C.I. = 42.2 ± 0.4 dB), 11.0 dB to 36.3 dB for the 100 Hz harmonic (mean ± 95% C.I. = 30.5 ± 0.4 dB), and 6.6 dB to 28.9 dB for the 150 Hz harmonic (mean ± 95% C.I. = 23.5 ± 0.3 dB). Depending on NL conditions and source depths, the maximum possible range of detection for the each hour ranged from 11 m to 1592 m, 0 m to 374 m, and 0 m to 132 m for the 50 Hz, 100 Hz, and 150

Hz harmonics, respectively, with a tendency for increased detection ranges with increasing source depth with the exception of the 150 Hz frequency (Figure 2.7). Since source depths were unknown in the study, the mean of maximum detection ranges across source depths of 5 – 18 m was calculated for each hour and ranged from 30 m to 1412 m (mean ± S.E. = 683 ± 15 m), 2 m to 298 m (mean ± S.E. = 100 ± 3 m), and 1 m to 92 m (mean ± S.E. = 32 ± 1 m) for the 50 Hz, 100 Hz, and 150 Hz harmonics, respectively (Figure 2.8).

Maximum detection ranges per hr of the first three harmonic frequencies varied as function of time and ambient NL, resulting in a nonstationary ability to detect different numbers of males present within fixed territories at the site (Figure 2.4; Figure 2.8). Given the spatial distribution of males and variable detection ranges of the 100 Hz harmonic (i.e., 2 m to 298 m), between 2 and 5 males accounted for the sounds detected by the analyst in any given hour. After calibrating the number of sounds detected per hr by dividing by the number of males detectable, estimated sound production rates per individual male confirmed diel patterns in acoustic behaviors that increased in the 1600 – 1800 hours and revealed differences in the total amount of sounds produced per hr across days (Figure 2.9).

Comparisons of sound production rates with rates of observed behaviors and females encountered during 1600 – 1800 hours indicated that time of day and rates of spawning rushes and females encountered were important predictors of the number of sounds produced by males within a given hour (Figure 2.10). Rates of females encountered and spawning rushes were positively correlated with rates of sound production (Spearman Rank; females encountered: $r_s(38) = 0.52$, $p < 0.001$; spawning rushes: $r_s(38) = 0.48$, $p = 0.002$), while head shakes and burst rises were not correlated with rates of sound production (Spearman Rank; head shakes: $r_s(38) = 0.01$, $p = 0.95$; burst rises: $r_s(38) = 0.29$, $p = 0.07$). Rates of spawning rushes explained more of the variability in sound production rates ($R^2 = 0.35$; AIC = 293) in comparison to rates of females encountered ($R^2 = 0.28$; AIC = 297). Random Forest analysis explained 41% of the variance in model predictions and ranked hour, spawning rushes, and females encountered as the most important predictor variables of sound production rates (Figure 2.11).

EFFECTIVE COMMUNICATION RANGES

Across all levels of ambient NL during the study, TL at SE = 0 for Gulf grouper hearing ranged from 6.1 dB to 32.3 dB (50 Hz), 0 dB to 16.9 dB (100 Hz), and 0 dB to 10.7 dB (150 Hz), corresponding to maximum communication ranges of 0 m to 192 m, 0 m to 13 m, and 0 m to 5 m (Table 2.2), respectively, that were dependent on source and receiver depths (Figures 2.12 – 2.14). On average, communication ranges increased with depth. For the 100 Hz and 150 Hz harmonics, additional increases in communication ranges were estimated for shallower depths. After calculating the mean of communication ranges across all source and receiver depths under the mean ambient NL per hr conditions measured during the study, the mean effective communication ranges for the three harmonic frequencies were estimated to be 20.4 m (50 Hz), 0.8 m (100 Hz), and 0.1 m (150 Hz). However, communication ranges varied throughout the study and were dependent on ambient NL (Table 2.2).

DISCUSSION

The results of this study demonstrate that for fish species with established numbers and distributions of territorial sound producers, such as males, environmentally calibrated counts of detected sounds can correspond to changes in rates of sound production that are indicative of spawning activity and relative female abundance. The incorporation of acoustic propagation modeling and measures of SL and DT permitted calculations of the detection ranges of sounds as a function of time and initial estimates of the effective communication ranges of Gulf grouper at the site. By accounting for the number of males present within detection ranges on an hour by hour basis, rates of sound production per individual were estimated to provide a more calibrated description of temporal patterns in acoustic behaviors that were independent of ambient NL variability and subsequently more appropriate for comparisons between behaviors and relative female abundance (Parks *et al*., 2011; Marques *et al*., 2013). The mean effective communication ranges of the first three harmonics were less than 21 m on average, which corroborate the assumed function of short range communication during male reproductive displays targeted towards females in close proximity (Rowell *et al*., *in review*).

For each of the three harmonic frequencies in the fish sounds, temporal patterns of ambient NL largely dictated the temporal variability in the ranges from which each frequency could be detected by human analysts within spectrograms and by conspecifics, highlighting the importance of understanding the impacts of NL when interpreting measures of detected sounds (Mellinger *et al*., 2007; Zimmer *et al*., 2008; Helble *et al.*, 2013b) and assessing communication abilities of fishes (Radford *et al*., 2014). During the study, increases in ambient NL at the three frequencies resulted in part from noise generated by localized boating activity and SCUBA diving associated with surveys and dive tourism. Thus, the detection ranges and effective communication ranges estimated in the study were largely affected by human activity and may not be representative of other time periods or sites (Wahlberg &

Westerberg, 2005; Wysocki *et al*., 2007). Biological sources and weather patterns affected hourly NL less than anthropogenic sources during daytime hours but likely was responsible for the variability measured when boat and SCUBA activity was absent (Wenz, 1962). As the mean hourly detection ranges of the second harmonic frequency used to detect Gulf grouper sounds varied by up to 296 m, within each hour the sounds detected were estimated to originate from between 2 – 5 males. Therefore, patterns in the numbers of sounds detected were driven by both acoustic activity and different numbers of males within the detection range of the acoustic recorder, as well as variability in NL. Future efforts aimed at documenting behavioral activity and abundance should incorporate detection theory, acoustic propagation modeling, and ambient noise analysis to account for the environmental effects on the detection of sounds (Stafford *et al*., 2007; Simard *et al*., 2008; Helble *et al*., 2013a). With such knowledge, rates of sound production can be properly estimated as a function of time or space, which as demonstrated in this study can differ significantly from raw numbers of sounds detected.

As Gulf grouper produce sounds directed towards females during head shakes, burst rises, and spawning rushes during evening hours (Rowell *et al*., *in review*), it was hypothesized that rates of sound production would vary and be indicative of all behaviors, which accordingly increase with female abundance prior to sunset. While hour of day was an important predictor of sound production rates, correlation and random forest analyses indicated that rates of spawning rushes and females encountered were more important correlates than head shakes and burst rises. Observations that sounds are not always produced during head shakes and burst rises may explain reductions in predictor importance (Rowell *et al*., *in review*). Our findings corroborate previous studies that have assumed changes in levels of sounds detected relate to changes in rates of fish sound production (Schärer *et al*., 2012; Bertucci *et al*., 2015) and support previous assumptions that levels of recorded sounds are indicative of reproductive activity (Locascio & Mann, 2011a; Montie *et al*., 2016, 2017) and the relative abundances of both sexes if properly calibrated (Mellinger *et al*., 2007; Rowell *et al*., 2012; Rowell *et al*., 2017). However, as some species produce sounds within multiple behaviors, the behavioral contexts of sounds need to be fully understood prior to making such inferences (Mann & Lobel, 1998; Vasconcelos *et al*., 2010; Tricas & Boyle, 2014).

The effective communication ranges of the three harmonic frequencies coupled with behavioral observations indicated, like other fish species, male Gulf grouper produce low-amplitude sounds in order to communicate over short distances when courting females within distances of 10 m (Simões *et al*., 2008; Kierl & Johnston, 2010; van der Sluijs *et al*., 2011). Depending on source and receiver depths, the estimates of this study found that under mean NL conditions, females need to be within a range of 32 m, 3 m, and 1 m to detect the first three harmonic frequencies, respectively. Unlike some species that may produce sounds to attract conspecifics to spawning locations (Brantley & Bass, 1994; Erisman & Rowell, 2017), territorial species like Gulf grouper may produce sounds that are detectable only over short distances in order to court specific females within their territories and successfully complete spawning bouts (Kierl & Johnston, 2010; van der Sluijs *et al*., 2011). For species that spawn in pairs within lek-like mating systems, males compete to maintain females within their territories and likely express fitness through visual displays and sound production (Myrberg *et al*., 1986; Lobel & Mann, 1995; Hutchings *et al*., 1999). In such mating systems, females select mates; thus, male Gulf grouper may produce sounds to maintain individual females within their territories, resulting in successful courtship and spawning (Mann *et al.*, 1997; Verzijden *et al.*, 2010; Vasconcelos *et al.*, 2012).

Unknowns about the hearing capabilities of Gulf grouper contribute a large degree of uncertainty into the estimated effective communication ranges presented in this study. As the DT of adult groupers have not been assessed, the selection of a DT of 27 dB from another species within the order of Scorpaeniformes (Motomatsu *et al.*, 1998) may underestimate the hearing capabilities of Gulf grouper. Future efforts that examine hearing thresholds in groupers in terms of acoustic pressure, acoustic particle motion, and hydrodynamic flow associated with sound may reveal different communication ranges from those estimated in this study. As fishes can sense sound as pressure fluctuations through swim bladder and otolith pathways (Popper & Fay, 1999; Braun & Grande, 2008), propagation loss was modeled for acoustic pressure in this study. However, otoliths and lateral lines of fishes can detect and localize sources of acoustic particle motion and hydrodynamic flow (Fay & Simmons, 1999; Webb *et al.*, 2008b; Zeddies *et al.*, 2012). Particle motion and hydrodynamic flow can differ significantly from pressure at short ranges from the source (i.e., the near field) and in multi-path environments at all ranges, and their contributions to communication in fishes is increasingly being appreciated (Tricas *et al.*, 2006; Radford *et al.*, 2012; Popper *et al.*, 2018). Given the short communication ranges of acoustic pressure estimated in this study coupled with increasing knowledge about different ‘hearing’ mechanisms in fishes, Gulf grouper may be able to detect acoustic particle motion and hydrodynamic flow generated by sounds over ranges different than pressure through accessory organs and pathways (Radford *et al.*, 2012; Higgs & Radford, 2013; Popper *et al.*, 2018). Thus, continued efforts are needed to fully understand the capabilities, mechanisms, and relevance of short range communication across the diverse assemblages of fishes (Braun & Grande, 2008).

The documentation of changes in sound production rates in Gulf grouper that are driven by hour of day, spawning activity, and relative female abundance contributes to a growing body of studies that demonstrate that the passive acoustic monitoring of fish sounds during reproductive periods can be used to estimate patterns of spawning and abundance (Monczak *et al*., 2017; Rowell *et al*., 2017). However, a number of other factors likely influence rates of sound production that were not examined in this study. The inclusion of acoustic propagation modeling, ambient noise analyses, and estimates of detection ranges in future studies will facilitate an enhanced understanding of acoustic behaviors through estimations of sound production rates (or fish abundance) from received sound levels. For species like Gulf grouper that apparently produce sounds to communicate information, increasing noise levels may degrade behavioral responses and functioning (Slabbekoorn *et al*., 2010; van der Sluijs *et al*., 2011; Stanley *et al*., 2017). Therefore, the impacts of different noise levels and different noise types on the communication abilities and reproductive success of fishes should be considered.

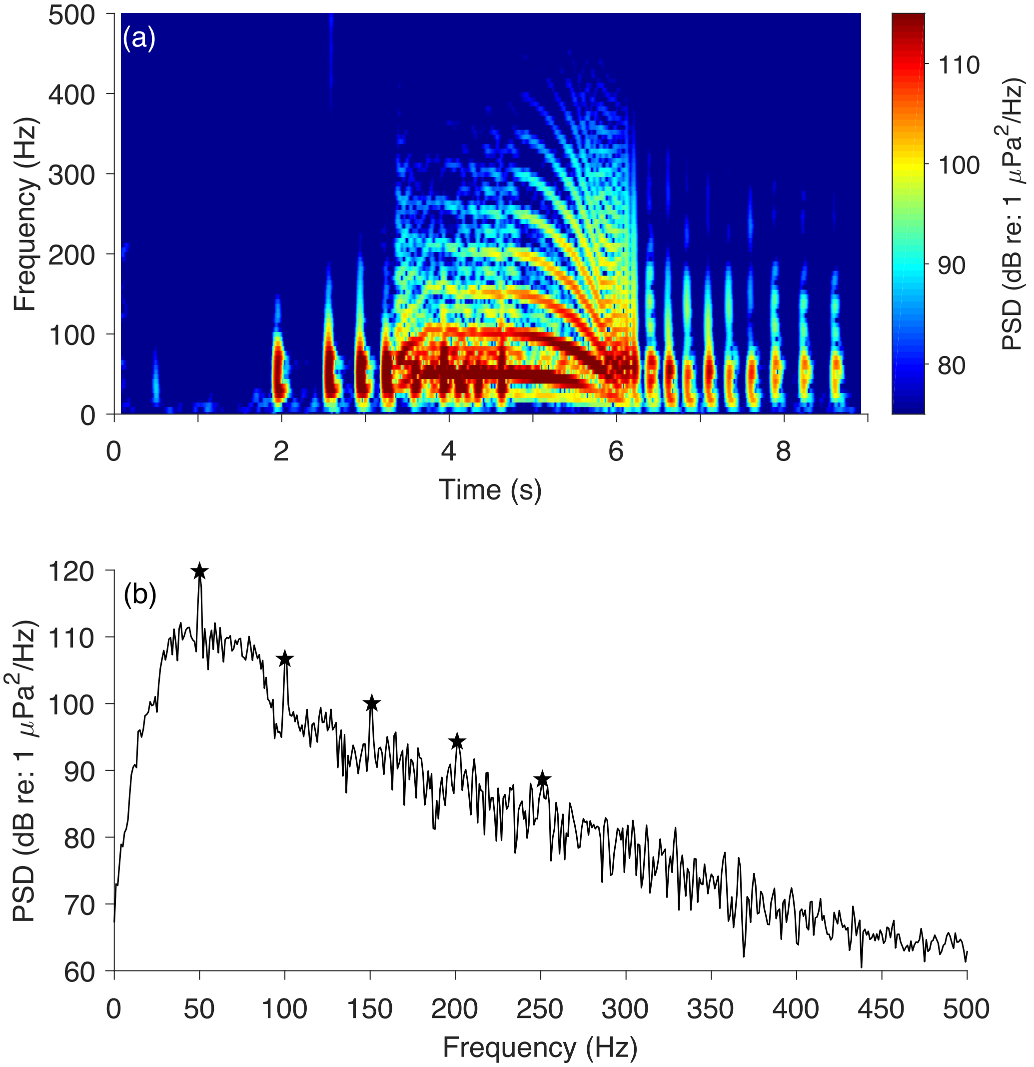


Figure 2.1. (a) Spectrogram of a sound produced by Gulf grouper (*Mycteroperca jordani*) during reproductive periods depicting the frequency composition of the sound as a function of time. (b) Pressure spectral density levels (PSD) of the sound with the dominant frequencies of five harmonics at 50 Hz, 100 Hz, 150 Hz, 200 Hz, and 250 Hz indicated with stars.

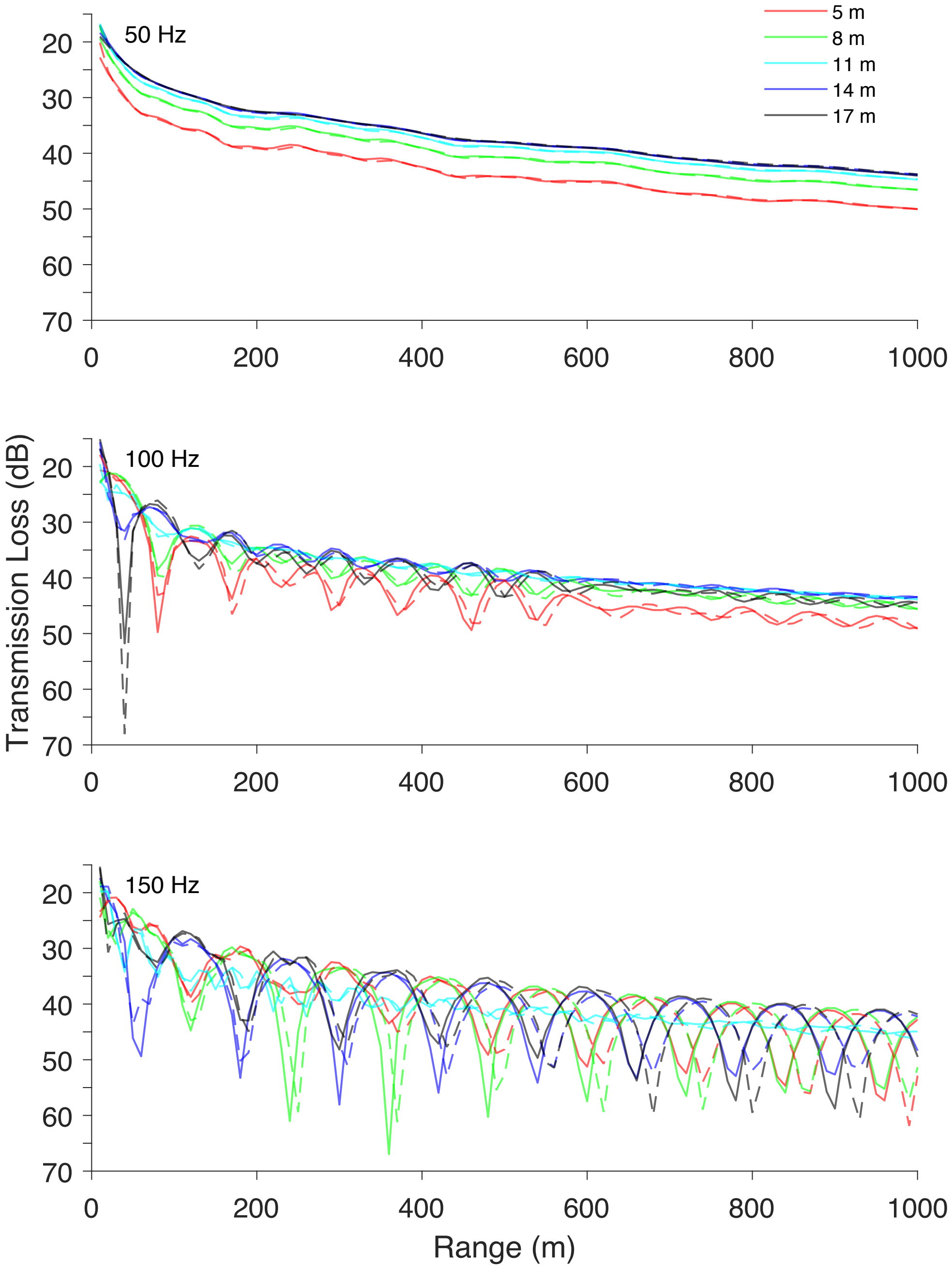


Figure 2.2. Transmission loss (TL; dB) of 50 Hz, 100 Hz, and 150 Hz signals to a receiver at 17 m depth as a function of range for source depths of 5, 8, 11, 14, and 17 m. Solid lines were estimated with the Kraken normal mode acoustic propagation model; dashed lines were estimated with the RAMGeo parabolic equation acoustic propagation model.

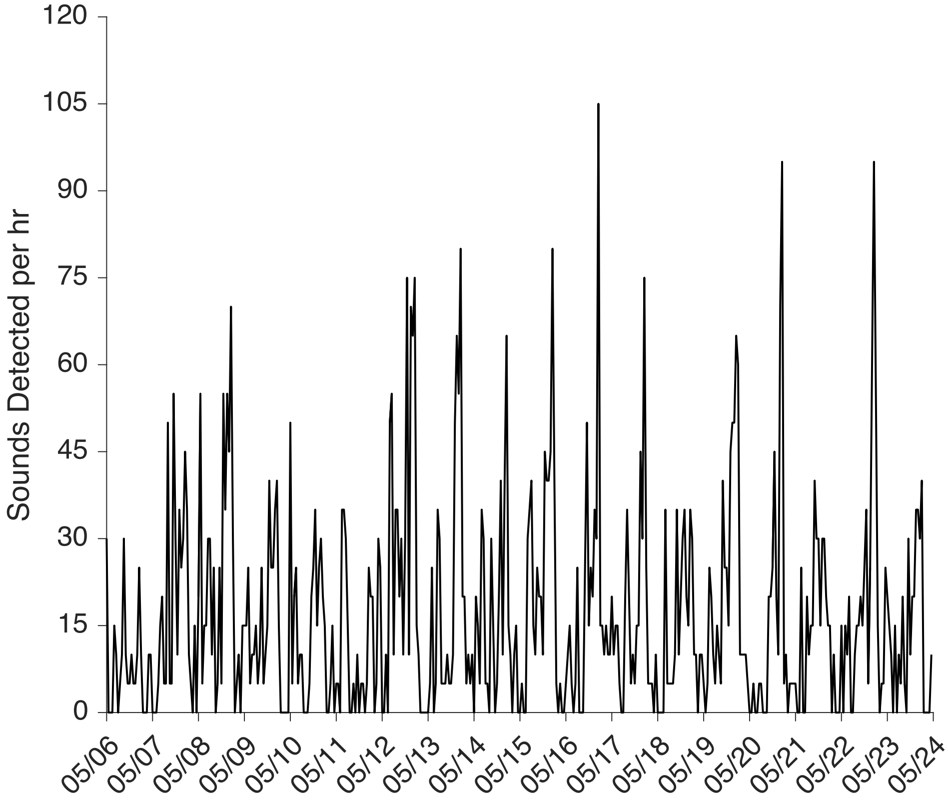


Figure 2.3. Time series of the number of Gulf grouper (*Mycteroperca jordani*) sounds per hr detected by an analyst from spectrograms. Only sounds with the first two harmonics detected were included in the counts.

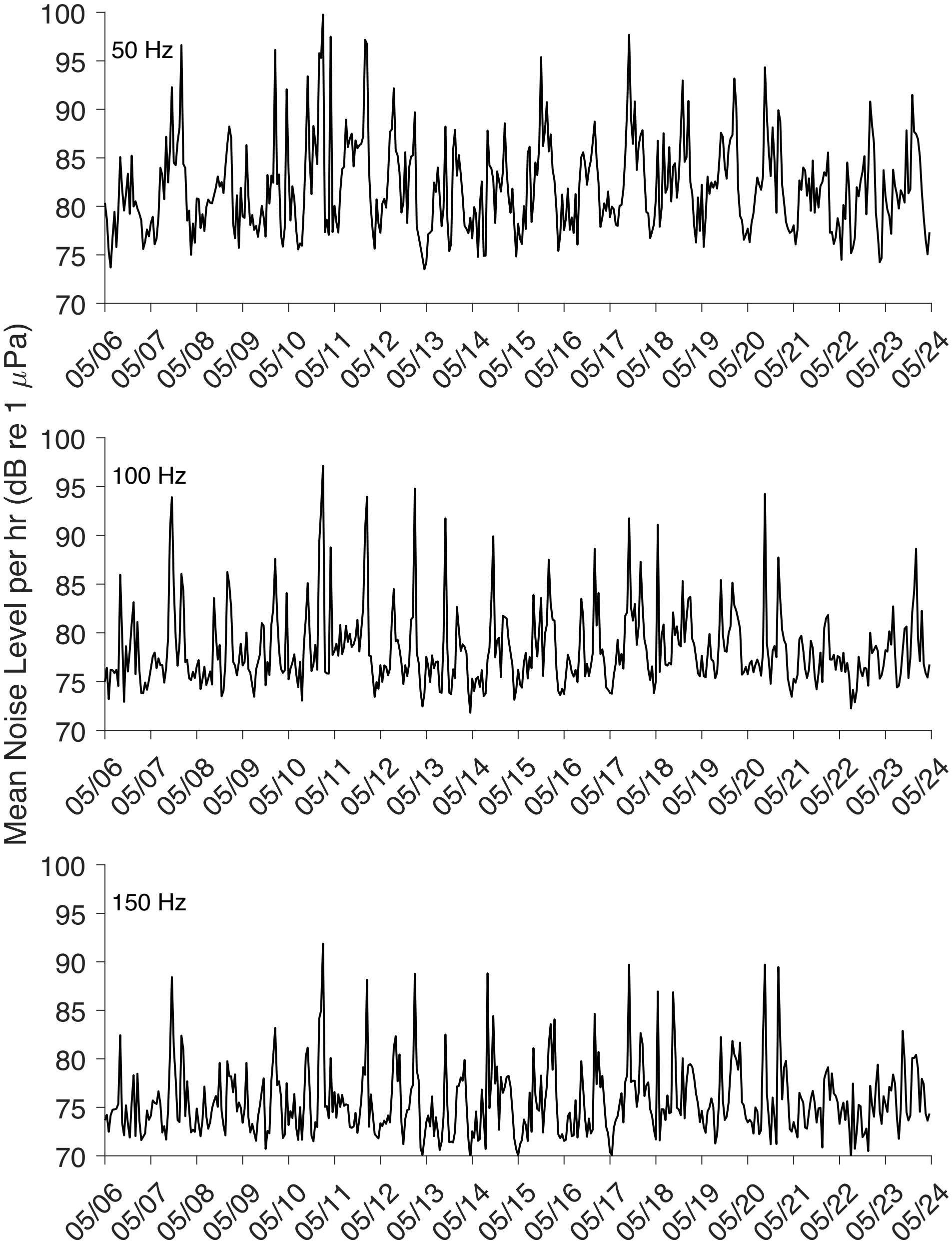


Figure 2.4. Time series of mean noise level (NL) per hr integrated over a 1 Hz-wide band centered at 50 Hz, 100 Hz, and 150 Hz for the duration of the study period.

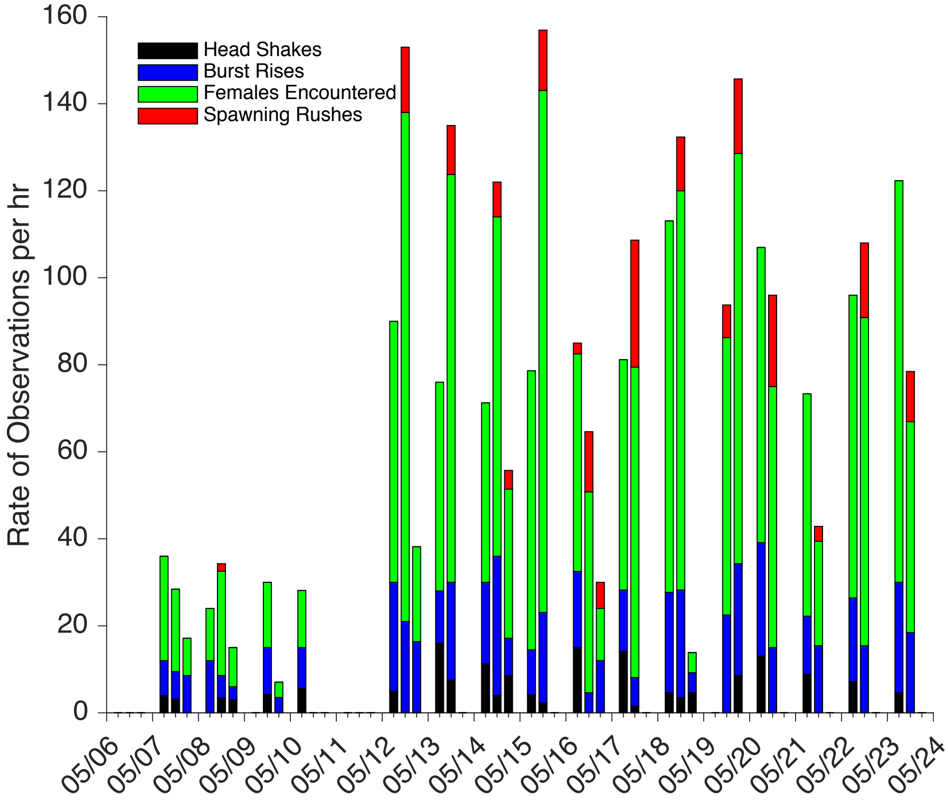


Figure 2.5. Rates of head shakes, burst rises, females encountered, and spawning rushes per hr observed by divers during evening surveys (1600 – 1800 hours MST) from 6 – 23 May 2017. Each bar represents one hour of observations; 1600, 1700, and 1800 MST are indicated with minor tick marks. Surveys were not conducted during all hours on all days. No surveys were conducted on 11 May 2017.

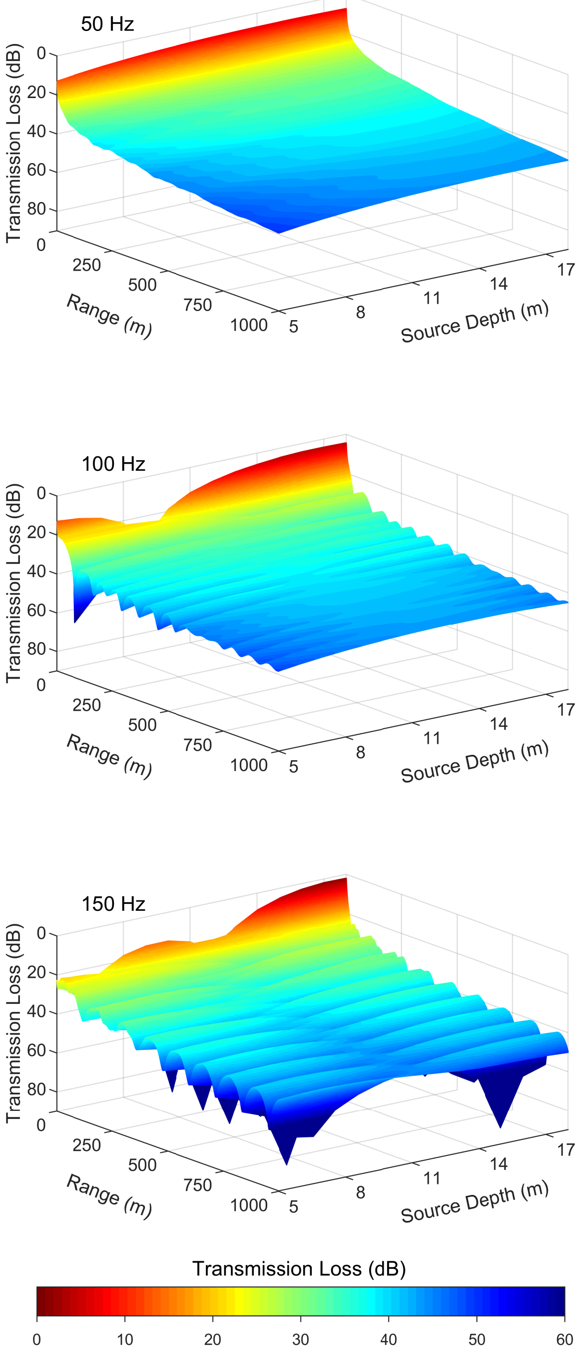


Figure 2.6. Estimates of transmission loss (TL; dB) as a function of range and source depth over the interval 5 – 8 m to a receiver at 17 m depth for 50 Hz, 100 Hz, and 150 Hz signals. Estimates were generated with Kraken.

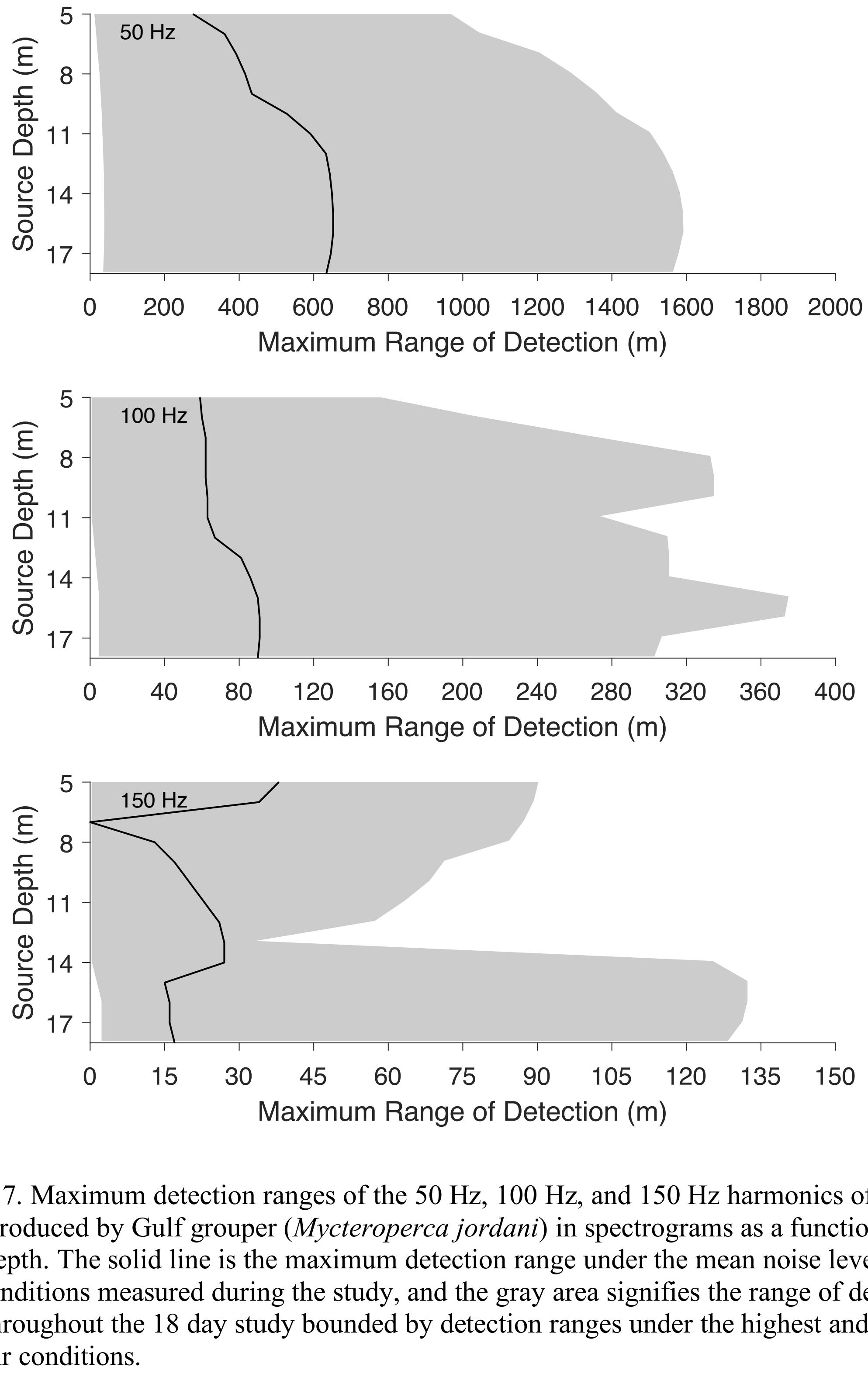


Figure 2.7. Maximum detection ranges of the 50 Hz, 100 Hz, and 150 Hz harmonics of sounds produced by Gulf grouper (*Mycteroperca jordani*) in spectrograms as a function of source depth. The solid line is the maximum detection range under the mean noise level (NL) per hr conditions measured during the study, and the gray area signifies the range of detection ranges throughout the 18 day study bounded by detection ranges under the highest and lowest NL per hr conditions.

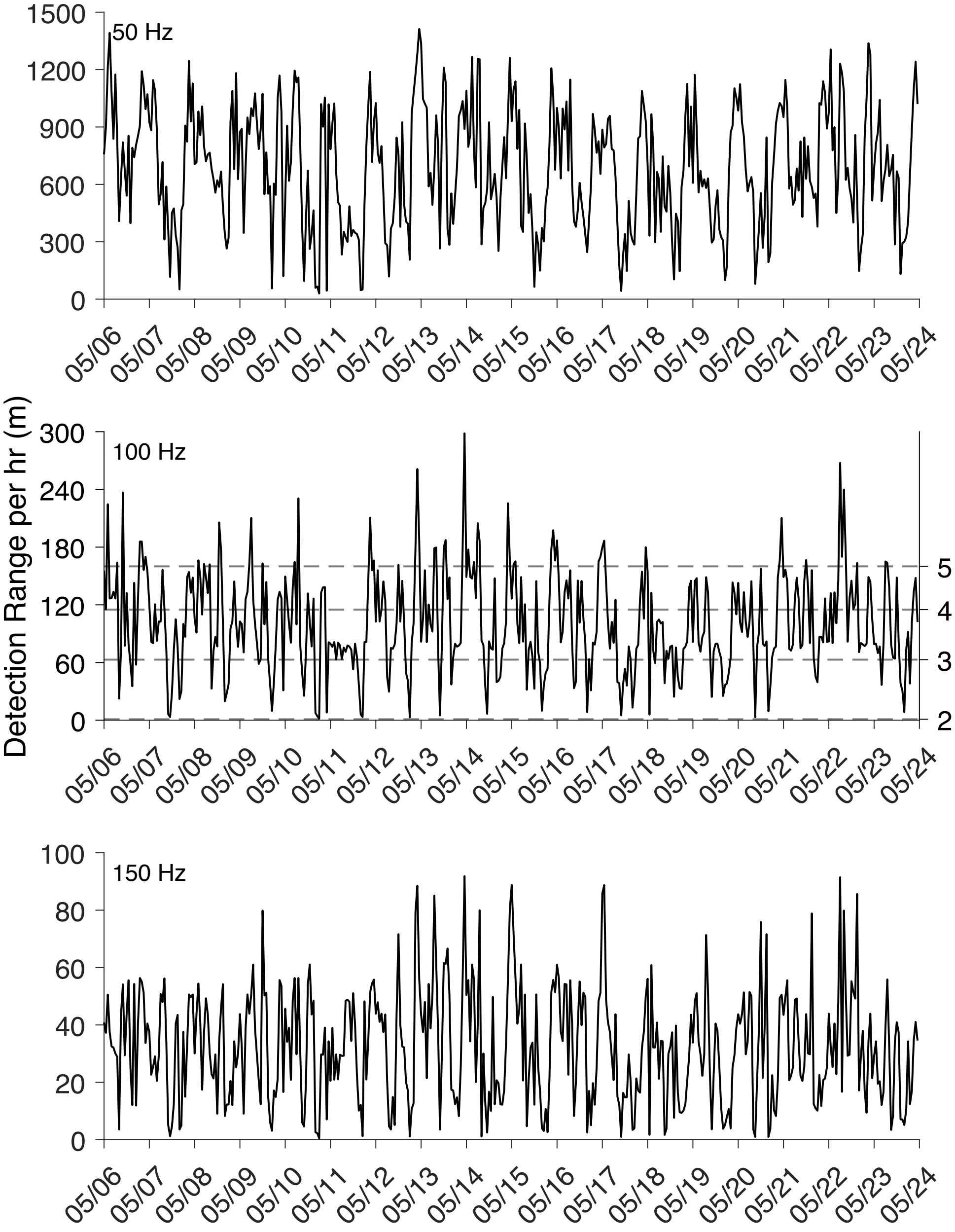
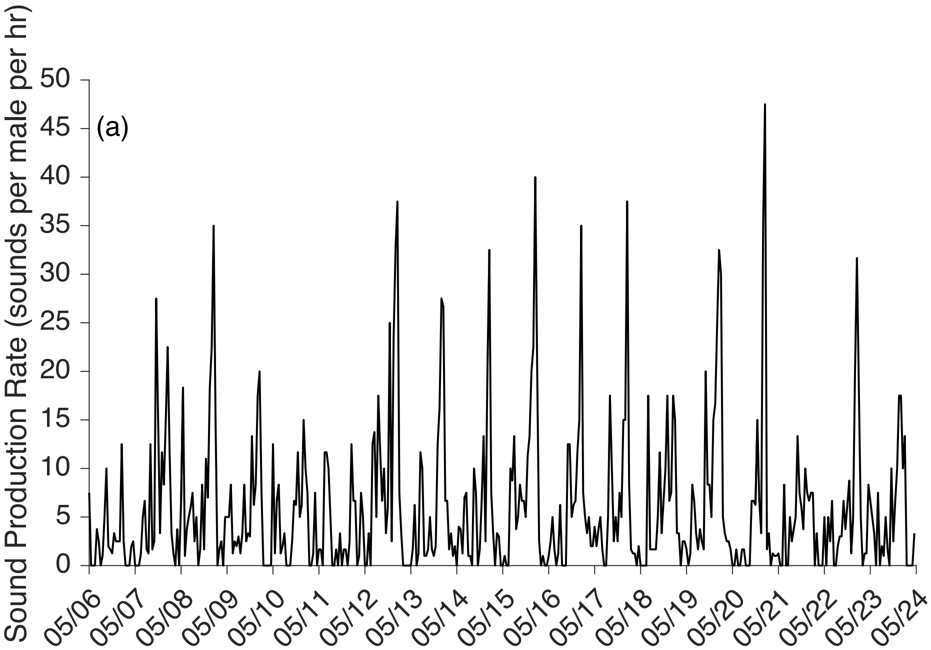


Figure 2.8. Mean detection range per hr of the 50 Hz, 100 Hz, and 150 Hz harmonics of sounds produced by Gulf grouper (*Mycteroperca jordani*) for human analysts examining the spectrograms. Fluctuating detection ranges of the 100 Hz harmonic resulted in hourly differences in the number of males from which sounds could be detected, ranging from 2 – 5 males per hr.



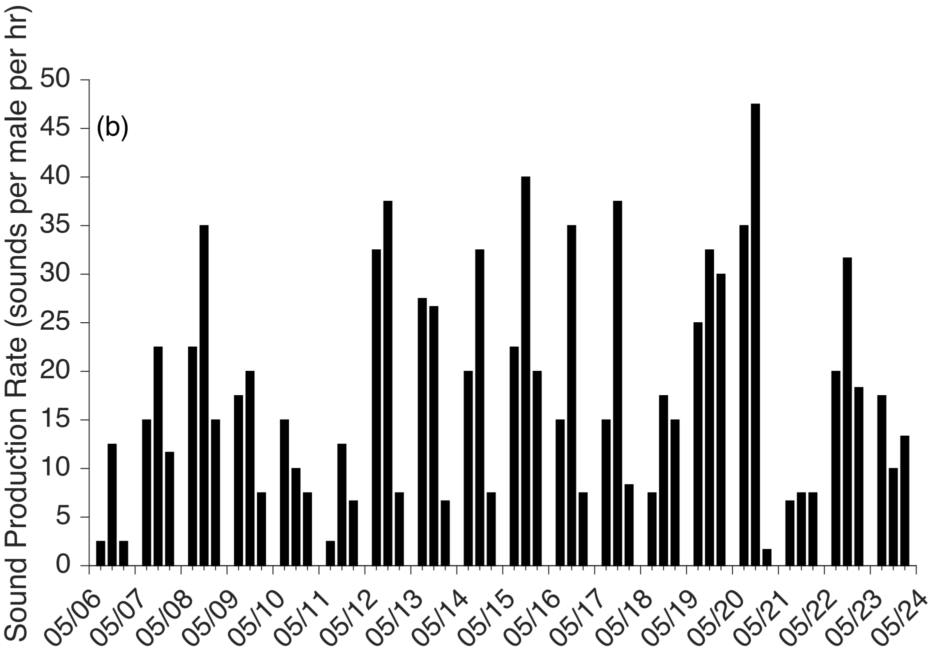


Figure 2.9. (a) Rates of sound production per male per hr estimated by dividing the number of sounds detected per hr by the number of males present within the detection range per hr. (b) Sound production rates within the observational survey hours; minor tick marks = 1600, 1700, and 1800 MST.

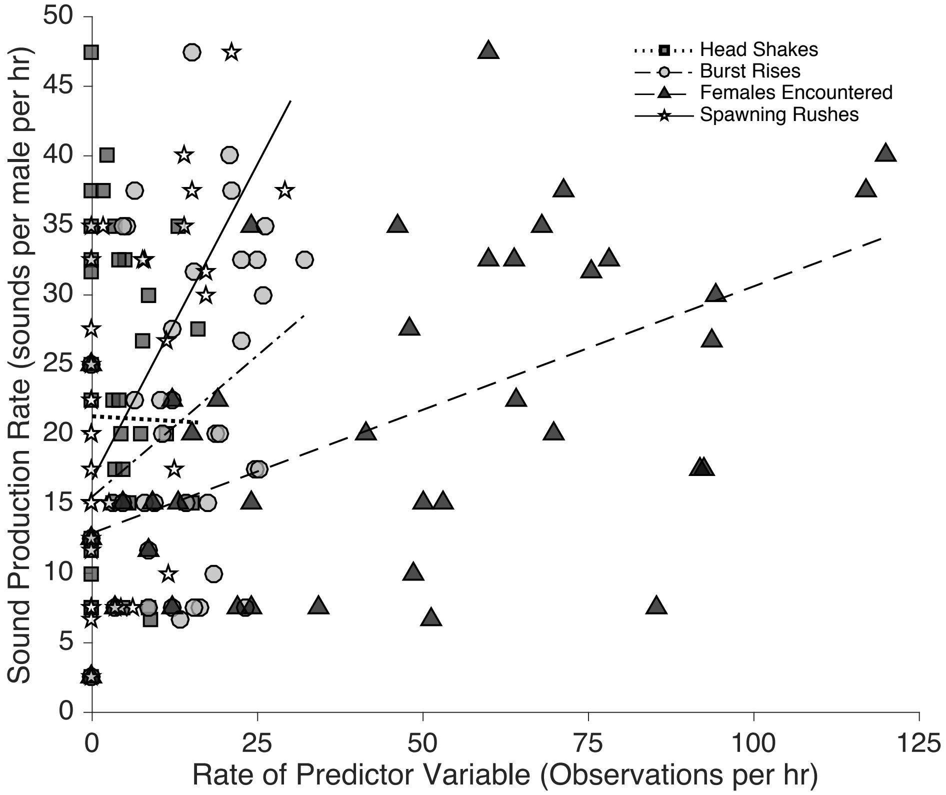


Figure 2.10. Relationships between hourly rates of sound production per male and observations of behaviors and females from 1600 – 1800 MST. Rates of females encountered and spawning rushes were positively correlated to rates of sound production. Regression lines were fitted with generalized linear models.

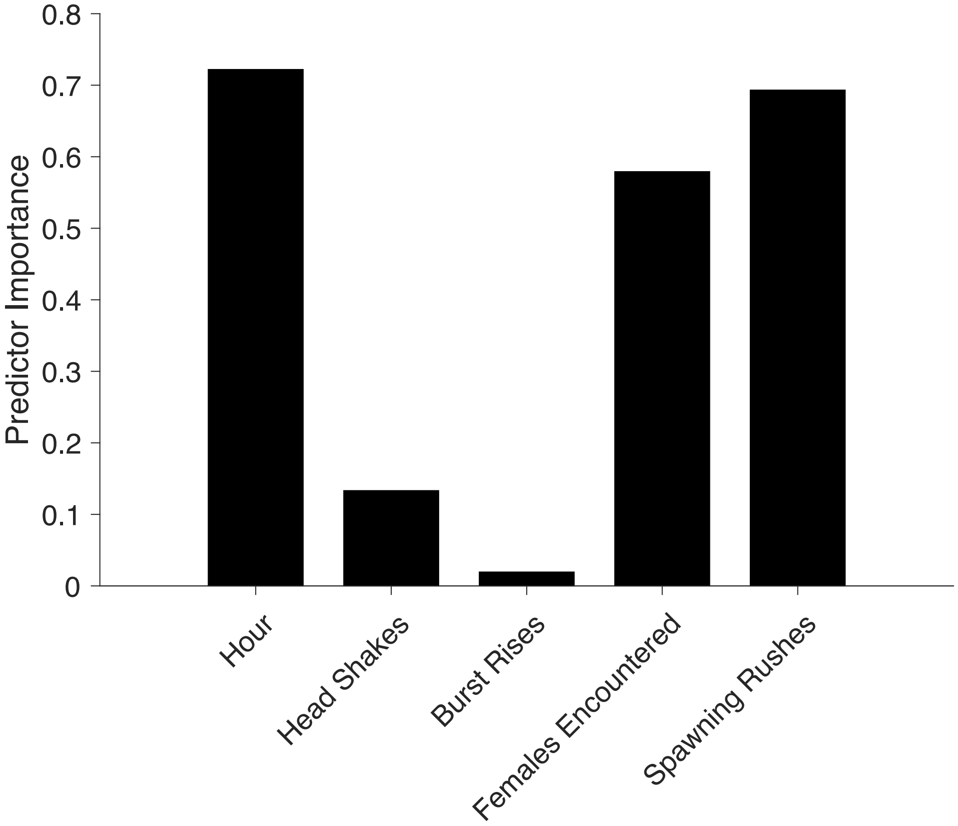


Figure 2.11. Relative importance of hour of day and rates of head shakes, burst rises, females encountered, and spawning rushes in predicting rates of sound production estimated with the Random Forest analysis.

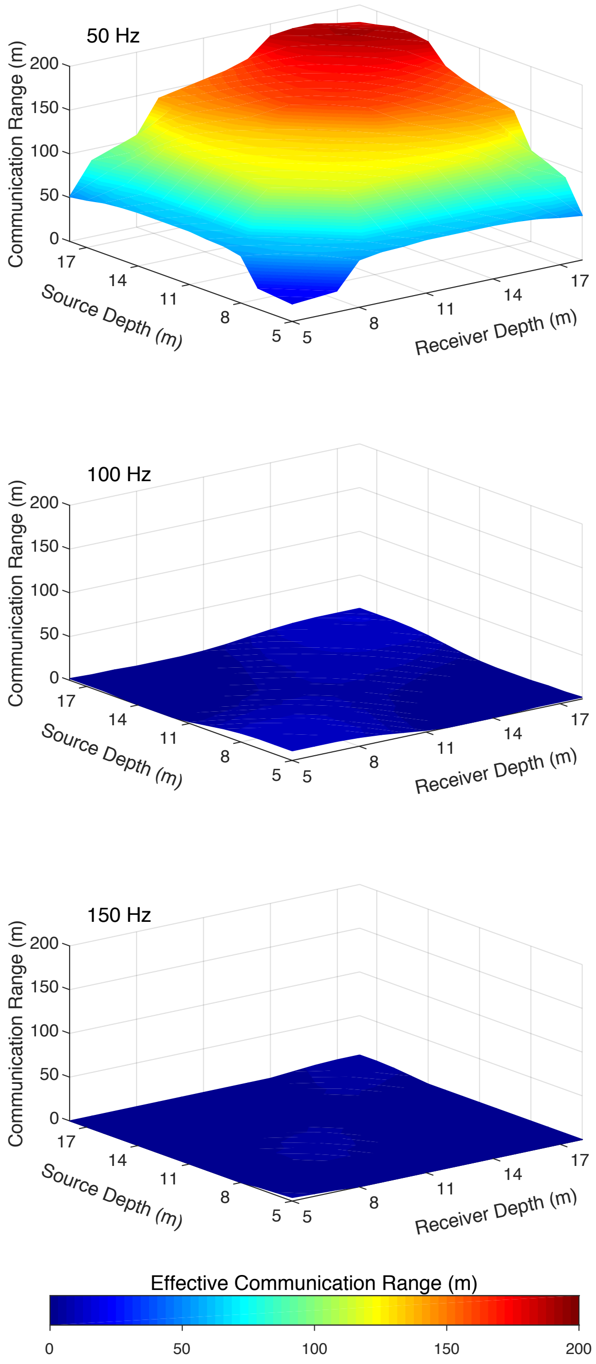


Figure 2.12. Estimated effective communication range of Gulf grouper (*Mycteroperca jordani*) as a function of source and receiver depth under the minimum noise level per hr conditions measured during the study. NL = 73.5 dB re 1 μPa (integrated over 1-Hz band centered at 50 Hz), 71.8 dB re 1 μPa (100 Hz), and 69.6 dB re 1 μPa (150 Hz).

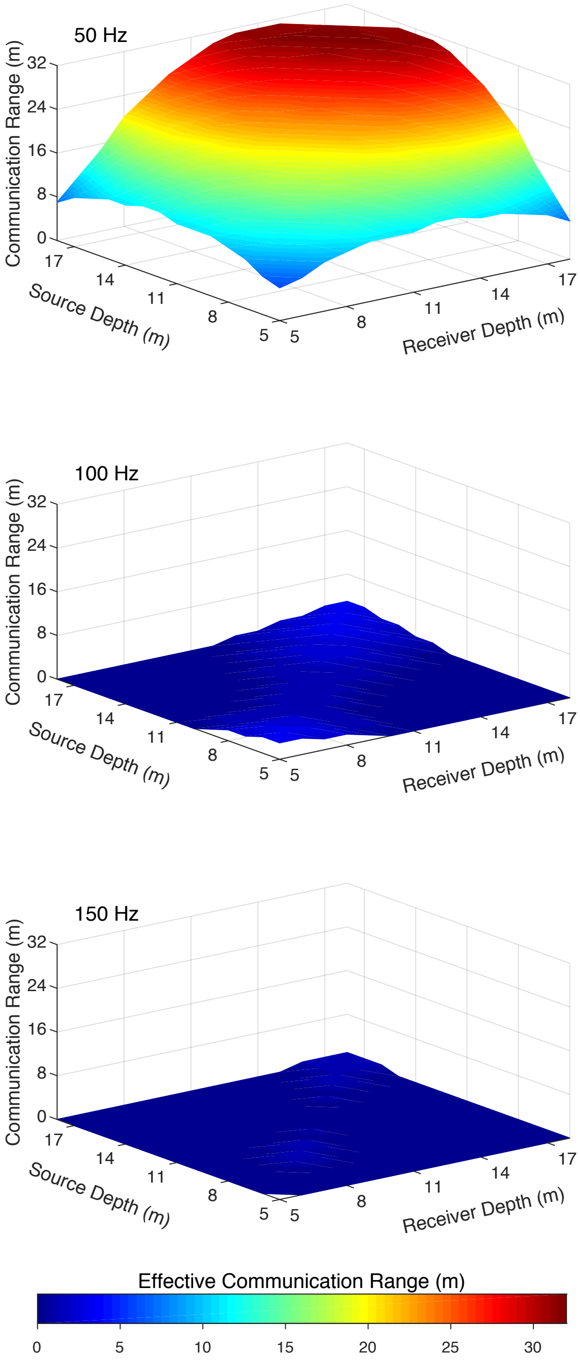


Figure 2.13. Estimated effective communication range of Gulf grouper (*Mycteroperca jordani*) as a function of source and receiver depth under the mean noise level per hr conditions measured during the study. NL = 83.4 dB re 1 μPa (integrated over 1-Hz band centered at 50 Hz), 79.5 dB re 1 μPa (100 Hz), and 76.5 dB re 1 μPa (150 Hz).

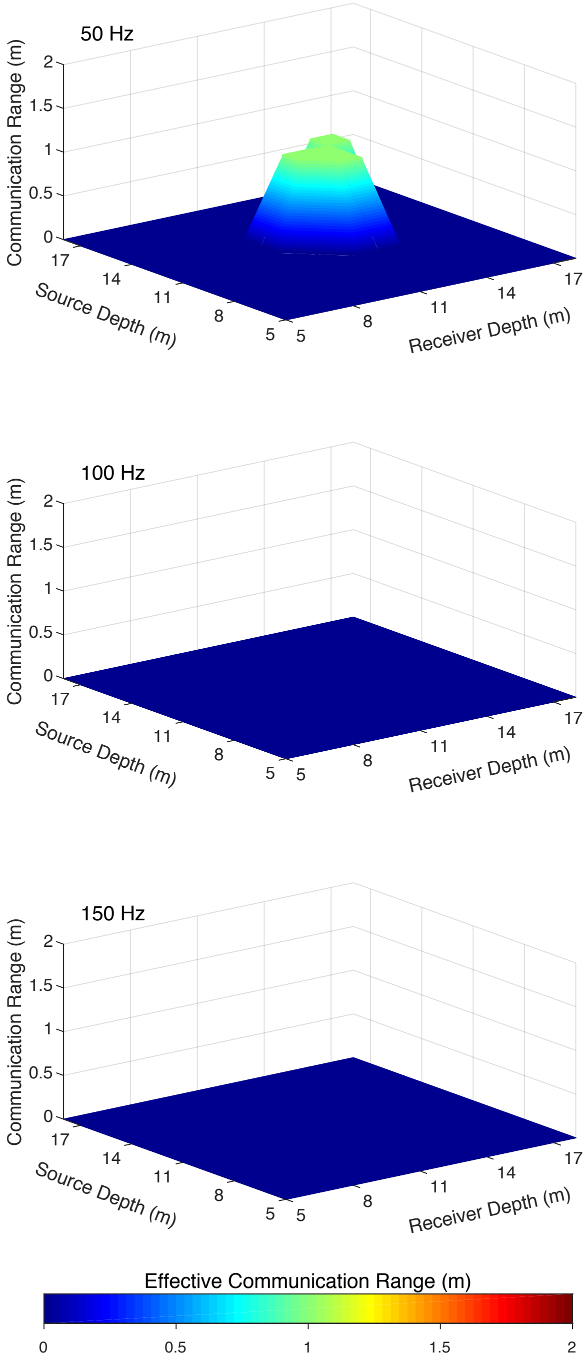


Figure 2.14. Estimated effective communication range of Gulf grouper (*Mycteroperca jordani*) as a function of source and receiver depth under the maximum noise level per hr conditions measured during the study. NL = 99.7 dB re 1 μPa (integrated in 1-Hz band centered at 50 Hz), 97.1 dB re 1 μPa (100 Hz), and 91.9 dB re 1 μPa (150 Hz).

Table 2.1. Values and sources of information of estimated range-and-azimuth-independent geoacoustic parameters used as input to the acoustic propagation models. The sediment layer and bottom half space were modeled as fluids (i.e., shear wave speed and attenuation were set to zero in both media).

Parameter	Value	Source of Information
Water Column		
Water depth (d_w)	18 m	*In situ* measurement
Sound speed (c_w)	1534 m s^{-1}	*In situ* CTD measurement
Density (ρ_w)	1023 kg m^{-3}	*In situ* CTD measurement
Sediment Interface		
Mean grain size (ϕ)	Coarse Sand 0.92	*in situ* sample
Sediment density (ρ_s)	2026 kg m^{-3}	Gardner et al. 1974, Hamilton 1978.
Compressional sound speed (c_p)	1836 m s^{-1}	Hamilton 1980
Attenuation of compressional waves (α_p; dB m^{-1})	$0.475 * f_{kHz}$	Hamilton 1980
Sediment		
Thickness (Z_{max})	50 m	Einsele and Niemitz 1982
Mean grain size (ϕ)	Coarse Sand 0.92	*in situ* sample
Sediment density (ρ_s; kg m^{-3}) as a function of compressional speed	$\rho_s = 230\left(\frac{c_p}{0.3048}\right)^{0.25}$	Gardner et al. 1974, Hamilton 1978
Compressional sound speed (c_p; m s^{-1}) as function of sediment depth (Z_{km})	$c_p = 1000 * (1.836 + 1.304Z - 0.741Z^2 + 0.257Z^3)$	Hamilton 1980
Attenuation of compressional waves (α_p; dB m^{-1}) as a function of sediment depth (Z_m)	$\alpha_p = k_p * Z^{\left(\frac{-1}{6}\right)} * f_{kHz}$	Hamilton 1980
Basalt half space		
Density (ρ_b)	2700 kg m^{-3}	Christensen and Salisbury 1975
Compressional sound speed (V_p)	5300 m s^{-1}	Christensen and Salisbury 1975, Hamilton 1980
Attenuation of compressional waves (α_p; dB m^{-1})	$\alpha_p = k_p * f_{kHz}$	Hamilton 1980

Table 2.2. Effective communication ranges at the dominant frequencies of three harmonics of sounds produced by Gulf grouper (*Mycteroperca jordani*) in three ambient noise conditions that correspond to the minimum, mean, and maximum noise levels (NL) per hr during 18 days of acoustic monitoring. Ambient NL were integrated over 1 Hz-wide bands. Estimates for all source and receiver depths of 5 – 18 m were averaged to estimate mean communication ranges. The minimum and maximum ranges correspond to the minimum and maximum of all individual source-receiver depth estimates.

Ambient NL (dB re: 1 μPa)	Frequency (Hz)	Communication Range Mean ± S.E. (m)	Minimum (m)	Maximum (m)
73.5 dB	50 Hz	121.6 ± 3.2 m	20 m	192 m
83.4 dB	50 Hz	20.4 ± 0.5 m	6 m	32 m
99.7 dB	50 Hz	0.1 ± 0.02 m	0 m	1 m
71.8 dB	100 Hz	4.9 ± 0.3 m	0 m	13 m
79.5 dB	100 Hz	0.8 ± 0.1 m	0 m	3 m
97.1 dB	100 Hz	0 m	0 m	0 m
69.6 dB	150 Hz	1.1 ± 0.1 m	0 m	5 m
76.5 dB	150 Hz	0.1 ± 0.02 m	0 m	1 m
91.9 dB	150 Hz	0 m	0 m	0 m

ACKNOWLEDGEMENTS

The authors would like to thank D. Castro-Arvizu, J. J. Cota-Nieto, M. A. Steele, C. Biggs, B. Martinez-Guerrero, T. TinHan, A. Johnson, A. Ramirez-Valdez, M. Doane, E. Reed, and the staff of Cabo Pulmo Divers for field assistance, members of the Centro Para la Biodiversidad Marina y Conservación A.C., Comisón Nacional de Áreas Naturales Protegidas (CONANP), and Parque Nacional Cabo Pulmo for logistical and permitting support, and P. A. Hastings, B. X. Semmens, J. B. Shurin, and D. A. Demer for intellectual guidance. The project was funded in part by grants to O.A. and B.E.E. from the Walton Family Foundation, the David and Lucile Packard Foundation, and the World Wildlife Fund México.

Chapter 2, in part, is currently being prepared for submission for publication of the material. Rowell, Timothy J.; D'Spain, Gerald L.; Aburto-Oropeza, O.; Erisman, B.E. The dissertation author was the primary investigator and author of this material.

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CHAPTER 3

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

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Cite this article: Erisman BE, Rowell TJ. 2017 A sound worth saving: acoustic characteristics of a massive fish spawning aggregation. *Biol. Lett.* **13**: 20170656.
http://dx.doi.org/10.1098/rsbl.2017.0656

Received: 19 October 2017
Accepted: 28 November 2017

Subject Areas:
ecology, behaviour, environmental science, evolution

Keywords:
fish chorus, spawning aggregations, bioacoustics, soundscapes, marine conservation

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Marine biology

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

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Group choruses of marine animals can produce extraordinarily loud sounds that markedly elevate levels of the ambient soundscape. We investigated sound production in the Gulf corvina (*Cynoscion othonopterus*), a soniferous marine fish with a unique reproductive behaviour threatened by overfishing, to compare with sounds produced by other marine animals. We coupled echosounder and hydrophone surveys to estimate the magnitude of the aggregation and sounds produced during spawning. We characterized individual calls and documented changes in the soundscape generated by the presence of as many as 1.5 million corvina within a spawning aggregation spanning distances up to 27 km. We show that calls by male corvina represent the loudest sounds recorded in a marine fish, and the spatio-temporal magnitude of their collective choruses are among the loudest animal sounds recorded in aquatic environments. While this wildlife spectacle is at great risk of disappearing due to overfishing, regional conservation efforts are focused on other endangered marine animals.

1. Introduction

Many animals in aquatic environments produce loud sounds in association with reproduction, social interactions and other behaviours [1,2]. In many circumstances, numerous individuals within a particular area produce sounds simultaneously, and these group choruses can greatly alter the acoustic environment (soundscape) through marked, ephemeral amplifications in ambient noise levels [3–5]. The intensity, duration and other characteristics of these sounds are evolutionary products of the specific function they serve and the soundscape within which they have evolved [6,7]. Moreover, species-specific characteristics of sound production are useful and practical for monitoring the presence, abundance and activity patterns of aquatic animals for research, management and conservation [8,9].

The Gulf corvina (*Cynoscion othonopterus*) is a species of croaker (family: Sciaenidae) endemic to the Northern Gulf of California, Mexico. It is one of many marine fishes known to produce sounds in association with spawning [10,11], but their common acoustic behaviour is tightly linked to a unique reproductive pattern threatened by overfishing [12]. Each spring, all adults of the species migrate to one site, the Colorado River Delta in the uppermost portion of the Gulf to form one spawning aggregation of several million fish [13]. Spawning is synchronized with tidal and lunar cycles, occurring during the outgoing tides over a 3- to 4-day period before the new and full moons [11,13]. Male corvina produce sounds during spawning, and their collective mating choruses reverberate through the hulls of small, fibreglass fishing boats (pangas) and are audible to the naked ear. The intense sound levels can mask and overwhelm concurrent engine noise from fishing activity, enabling fishers to use the sound to easily locate and harvest fish

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Electronic supplementary material is available online at https://dx.doi.org/10.6084/m9.figshare.c.3949612.

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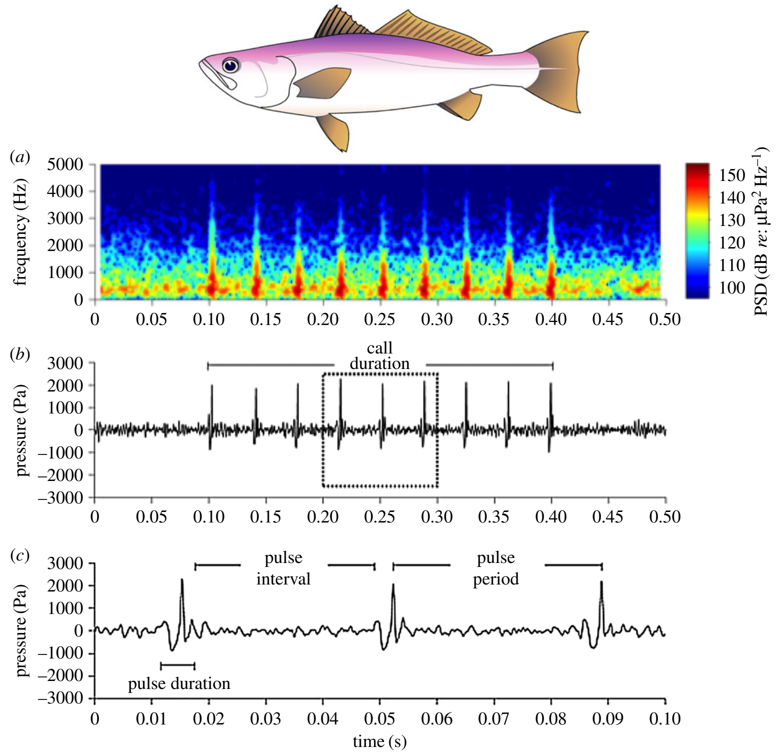

Figure 1. A single Gulf corvina call comprised nine pulses. The frequency composition of calls and choruses are depicted in a spectrogram (*a*), where the nine individual pulses overlay continuous background chorusing at frequencies below 2000 Hz. Oscillograms of the entire call (*b*) and a close-up of three pulses (*c*; boxed area shown in (*b*)) show the structure and high pressure levels (Pa) of pulses recorded. PSD, pressure spectral density. Fish illustration by L. G. Allen.

during spawning [11]. A single panga with one net can catch 2 tons of corvina within minutes, and the local fleet of 500 pangas harvests up to 5900 tons (2 million corvina) in 20 days of fishing each year, placing the species at great risk of collapsing [12,13].

We investigated the dynamics of sound production associated with corvina spawning to compare with the magnitude of noise produced by other marine animals. Our results identify the spawning sounds of male corvina as the loudest sounds recorded for a marine fish, loud enough to harm the hearing of other marine animals. The levels and spatial dimensions of sound produced by the entire aggregation coupled with the migration of an entire adult population to a single location constitutes a wildlife spectacle that merits increased protection.

2. Material and methods

We conducted 4 days of acoustic surveys of the corvina aggregation in the Delta during peak spawning periods in March and April 2014. Active acoustic (split-beam echosounder) surveys were comprised of transects across the Delta channel from a panga. Echosounder data were analysed to estimate the distribution and abundance of fish for each survey (electronic supplementary material). Passive acoustic (hydrophone) surveys of corvina sound production were conducted from a second vessel, in synchrony with the timing and location of echosounder transects, to estimate the magnitude and spatial distribution of received sound pressure levels (SPL; $\mathrm{dB_{rms}}$ *re*: 1 µPa) attributable to spawning corvina (electronic supplementary material). Additional sound measurements were made outside of the spawning period to document the mean ambient SPL in the absence of corvina sound production.

We isolated audio recordings of calls with high signal to noise ratios and choruses exceeding 150 $\mathrm{dB_{rms}}$ to characterize the individual calls and collective choruses of male corvina. Oscillograms of calls were generated to estimate call duration, pulses per call, pulse duration, pulse interval and pulse period. We isolated single pulses from calls to calculate three measurements of individual sound levels (dB measured as 0-to-peak (0-p), peak-to-peak (p-p) and rms) and estimate source levels (dB at 1 m) based on the maximum levels recorded. Pressure spectral density (dB *re*: 1 $\mu\mathrm{Pa}^2\ \mathrm{Hz}^{-1}$) curves of calls and choruses were generated to estimate their peak frequencies and 3 dB and 6 dB bandwidths (Hz), which describe the distribution of acoustic pressure as a function of frequency.

Fish densities and SPLs per transect were mapped to delineate the aggregation, spawning activity and chorusing. The spatial extent of each aggregation event was estimated by measuring the linear distance over which densities per transect were greater than two fish per 1000 m^3. The linear distance of SPLs exceeding 150 $\mathrm{dB_{rms}}$ was measured to estimate the spatial extent of pronounced spawning activity for each survey. As peak spawning and stable sound production rates occur over a predictable 2 h period [11], we calculated cumulative sound exposure levels

Table 1. Characterization statistics of Gulf corvina calls and choruses. Maximum values of pulse SPLs represent the minimum expected source levels (dB at 1 m) of Gulf corvina.

	mean	CI_{95}	*n*	min	max
calls					
call duration (ms)	389.2	373.7, 404.7	139	103.3	655.0
pulses per call	11.1	10.7, 11.5	139	4	20
pulse duration (ms)	5.5	5.4, 5.6	1520	2.4	9.5
pulse interval (ms)	32.6	32.4, 32.8	1381	17.1	80.1
pulse period (ms)	37.9	37.7, 38.1	1367	29.8	60.2
pulse SPL ($dB_{0\text{-}p}$ *re*: 1 μPa)	176.3	176.1, 176.5	1530	164.3	187.2
pulse SPL ($dB_{p\text{-}p}$ *re*: 1 μPa)	179.6	179.4, 179.8	1530	167.4	190.0
pulse SPL (dB_{rms} *re*: 1 μPa)	166.6	166.4, 166.8	1530	155.7	177.3
peak frequency (Hz)	384.3	372.8, 395.8	139	265	655
3 dB bandwidth (Hz)	4.9	4.5, 5.3	139	2	16
6 dB bandwidth (Hz)	7.6	7.0, 8.2	139	2	20
choruses					
peak frequency (Hz)	377.9	372.8, 383.0	182	314	475
3 dB bandwidth (Hz)	14.0	12.4, 15.6	182	3	64
6 dB bandwidth (Hz)	209.8	203.4, 216.2	182	27	322

(SEL_{cum}; dB *re*: 1 μPa^2 s) throughout the aggregations by integrating received levels recorded during this period. We used SEL_{cum} values, which estimate the total (i.e. additive) sound energy produced during the 2 h spawning event, to infer the potential impact of elevated acoustic exposure on marine mammals present at the site.

3. Results

Recorded calls consisted of a variable number of short, sequential pulses repeated over the duration of the call (figure 1; table 1). Pulses were comprised of low amplitude positive and negative peaks followed by a high amplitude positive peak and single-peak decay. The maximum SPLs of pulses recorded were 187.2 $dB_{0\text{-}p}$, 190.0 $dB_{p\text{-}p}$ and 177.3 dB_{rms}, which represent the minimum expected source levels of corvina given that the exact source range was not measured. The mean peak frequencies of calls and choruses were 384 Hz and 377 Hz, respectively.

The spawning aggregation and associated chorusing were distributed over distances of 9–27 km (mean = 16 km). Fish densities per transect ranged from 0.07 to 33.94 fish per 1000 m^3 (figure 2), and total abundance ranged from 522 201 (CI_{95} 387 606–673 620) to 1 551 729 (CI_{95} 1 253 025–1 988 297) fish per survey. Received sound levels ranged from 136.17 to 163.43 dB_{rms} or 6.43 to 148.51 Pa_{rms} ($n = 117$; figure 2) during surveys of the aggregation compared to 99.34 (CI_{95} 98.26–100.42) dB_{rms} outside the spawning period, indicating that corvina choruses elevated levels of the soundscape by 64.09 dB_{rms}. SEL_{cum} ranged from 179.57 to 201.98 dB during the peak spawning period.

4. Discussion

Estimated source levels of calls by male corvina represent the loudest sounds recorded in a marine fish and among the loudest animal sounds recorded in aquatic environments (table 2). However, the magnitude of the sounds produced by corvina are best described by the simultaneous chorusing of males within the larger spawning aggregation, which can extend up to 27 km in length along the main channel of the Delta and include 1.5 million individuals during a single spawning period. The chorus of the aggregation can elevate the local soundscape 21 times louder than ambient levels, making these unique acoustic behavioural events a true wildlife spectacle. Notably, our measurements likely underestimate the soundscape potential of the event, given the intense, persistent fishing activity that has greatly reduced the size of the adult population [12,13].

Hearing mechanisms and performance in animals can evolve in accordance with environmental acoustics, background noise and the range of detectable sounds that are biologically relevant [28]. Ambient noise levels in the Delta are known to be unusually high [29]. Therefore, the loud acoustic characteristics of corvina may represent an evolutionary adaptation that permits intraspecific communication in an inherently noisy environment, a phenomenon termed the Lombard effect [30]. Like other animals that engage in mass breeding choruses (e.g. crickets and frogs) [31], the loud sounds produced by male corvina during spawning may facilitate communication among potential mates in a noisy environment made even noisier by the synchronous chorusing of conspecifics (i.e. the ‘cocktail party problem’ [32]). We speculate that the magnitude of sound produced serves to coordinate the mass, brief, synchronous spawning activity of the entire adult population that occurs during the outgoing tide in highly turbid and turbulent waters that render visual cues much less effective.

The SEL_{cum} produced in 2 h by the corvina aggregation ranged from 179 to 202 dB and was concurrent with a 21-fold (i.e. 64 dB) increase in ambient sound; increases of 3 dB equate to a doubling of sound intensity. SEL_{cum} of 173–219 dB over a 24 h period can cause permanent hearing loss

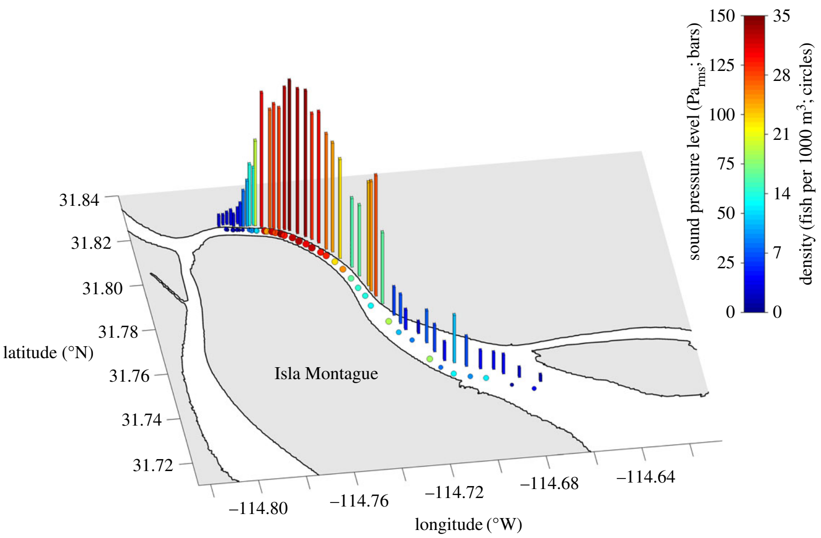

Figure 2. Map showing the distribution of fish densities (circles) and courtship sound levels (vertical bars) from acoustic surveys of Gulf corvina in the Colorado River Delta.

Table 2. Sounds produced by marine animals ranked by estimated source levels. Bold text identifies estimations made during this study.

species	source level and type (dB$_{rms}$ *re*: 1 μPa)	reference
sperm whale (*Physeter microcephalus*)	236, sonar click	Møhl *et al.* [14]
blue whale (*Balaenoptera musculus*)	189, call	Širović *et al.* [15]
fin whale (*Balaenoptera physalus*)	189, call	Širović *et al.* [15]; Weirathmueller *et al.* [16]
bowhead whale (*Balaena mysticetus*)	185, song	Tervo *et al.* [17]
Gulf corvina (*Cynoscion othonopterus*)	**177, call**	**Erisman & Rowell** [18]
humpack whale (*Megaptera novaeangliae*)	169, song	Au *et al.* [19]
black drum (*Pogonias cromis*)	165, call	Locascio & Mann [20]
humpback whale (*Megaptera novaeangliae*)	158, call	Dunlop *et al.* [21]
Nassau grouper (*Epinephelus striatus*)	143, call	Schärer *et al.* [22]
killer whale (*Orcinus orca*)	140, whistle	Miller [23]
bottlenose dolphin (*Tursiops truncates*)	138, whistle	Frankel *et al.* [24]
silver perch (*Bairdiella chrysoura*)	135, call	Sprague & Luczkovich [25]
oyster toadfish (*Opsanus tau*)	126, call	Barimo & Fine [26]
Bocaccio rockfish (*Sebastes paucispinis*)	113, call	Širović & Demer [27]

in cetaceans and pinnipeds, and temporary hearing loss in these animals can occur at SEL$_{cum}$ of 153–199 dB [33]. The frequency of sound produced by corvina falls within the range of hearing of pinnipeds and cetaceans, and SEL$_{cum}$ generated at the aggregation exceeded recommendations for daily (e.g. 24 h) exposure thresholds after only 2 h [33]. Therefore, it was surprising that adult California sea lions (*Zalophus californianus*) and dolphins were frequently observed concomitantly feeding in the area despite the potentially detrimental risk imposed by the elevated soundscape.

The corvina is endemic to the Northern Gulf of California, reproduces within an area that represents less than 1% of its species range, and faces imminent risk of a fishery and species collapse due to overfishing of its spawning aggregation and regulations that allow overfishing to persist [12,13]. A regional ban on commercial gill nets was recently implemented to protect two other endemic and endangered species, the vaquita porpoise (*Phoceona sinus*) and the Totoaba (*Totoaba macdonaldi*), but allows gill net fishing for corvina during spawning to continue [34]. Ironically, while sound has long been used to exploit corvina, sound production is highly correlated with fish abundance and can be used as a practical tool to estimate the population size, monitor the spawning population and set sustainable harvest limits [11]. Fish sounds are increasingly being valued for the information they convey about the biology, behaviour and population sizes of fishes, but the persistence of loud acoustic events, such as those created by corvina, into the future has intrinsic value worthy of conservation due to their uniqueness and warranted inclusion among wildlife spectacles.

Ethics. Research protocol was approved under UCSD IACUC ID no. S13240. Data were collected under CONANP permit no. CNANP-00-007.

Data accessibility. Data are available online at https://inport.nmfs.noaa.gov/inport/item/27628.

Authors' contributions. Both authors designed the study, collected field data, analysed the data, wrote the manuscript, agreed to be held accountable for the content therein and approved the final version of the manuscript.

Competing interests. We declare we have no competing interests.

Funding. Work was funded by the NOAA Ocean Acoustics Program.

Acknowledgements. We thank O. Aburto-Oropeza, J. Hyde, D. Demer and members of the Advanced Survey Technologies Program at SWFSC, G. D'Spain, J.J. Cota-Nieto, C. Lopez Sagastegui, J. Montañez Rivera, A. Montes Zamudio, J. Cabrera Rivera, E. Cabrera Rivera, Y. Flores, S. Freeman and CONANP (Mexico).

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APPENDICES

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-tec™, 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 (μPa_{rms}) and converted to decibels (dB_{rms} *re*: 1µPa), where $dB_{rms}\ re: 1\ \mu Pa = 20 * log_{10}(\mu Pa_{rms})$. All calculations were made within Matlab (The Mathworks®, USA). Sound pressure levels were mapped as dB_{rms} 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 dB_{rms}. As peak spawning and stable sound production rates occur over a predictable two-hour period [6], cumulative sound exposure levels (SEL_{cum}; dB *re*: 1 μPa^2-s) throughout the aggregations were calculated by integrating received levels recorded during this period. SEL_{cum} 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 (dB_{rms}) 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 dB_{rms} 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 μPa^2/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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ACKNOWLEDGEMENTS

Chapter 3, in full, is a reprint of the material as it appears in Biology Letters 2017. Erisman, Brad E.; Rowell, Timothy J. The dissertation author was the primary investigator and author of this material.

CHAPTER 4

Estimating fish abundance at spawning aggregations from courtship sound levels

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Estimating fish abundance at spawning aggregations from courtship sound levels

Received: 19 December 2016
Accepted: 27 April 2017

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Sound produced by fish spawning aggregations (FSAs) permits the use of passive acoustic methods to identify the timing and location of spawning. However, difficulties in relating sound levels to abundance have impeded the use of passive acoustics to conduct quantitative assessments of biomass. Here we show that models of measured fish sound production versus independently measured fish density can be generated to estimate abundance and biomass from sound levels at FSAs. We compared sound levels produced by spawning Gulf Corvina (*Cynoscion othonopterus*) with simultaneous measurements of density from active acoustic surveys in the Colorado River Delta, Mexico. During the formation of FSAs, we estimated peak abundance at 1.53 to 1.55 million fish, which equated to a biomass of 2,133 to 2,145 metric tons. Sound levels ranged from 0.02 to 12,738 Pa^2, with larger measurements observed on outgoing tides. The relationship between sound levels and densities was variable across the duration of surveys but stabilized during the peak spawning period after high tide to produce a linear relationship. Our results support the use of active acoustic methods to estimate density, abundance, and biomass of fish at FSAs; using appropriately scaled empirical relationships, sound levels can be used to infer these estimates.

Quantitative assessments of fish abundance form the basis of fisheries management and conservation strategies[1,2], but the complex migratory patterns, broad spatial distributions, life histories, and population dynamics of many fish species make it difficult to survey entire populations[3]. Some of these challenges are minimized if surveys are conducted where and when fish form large conspecific spawning aggregations[4]. Fish spawning aggregations (FSAs) occur at predictable times and locations, permitting surveys to estimate spawning stock abundance, biomass, and length and age distributions of regional populations[5,6].

Efforts to monitor FSAs and manage fishing intensity often rely on fisheries-dependent data (e.g., catch per unit effort) to estimate stock abundance[7]. However, these data can be insensitive to population declines since fish densities at FSAs may not change proportionally with abundance[8,9], making these methods inefficient for monitoring, assessments, and establishing quotas of total allowable catch. Fisheries-independent data often yield more accurate assessments of stocks[10], but methods such as mark-recapture, visual census, and trawls can be invasive, laborious, expensive, and inefficient or ineffective across the diverse environments where FSAs occur[11,12]. Therefore, the continued development and expansion of fisheries-independent methods to survey FSAs in challenging habitats are greatly needed to improve the quality of population assessments and manage vulnerable spawning stocks in a sustainable manner[6].

The adaptability of active acoustic methods to survey fishes in both deep[13] and shallow water habitats[14,15] enables assessments of populations across a spectrum of environments where constraints of depth and low visibility prevent the use of other methods. As a trusted source of fisheries-independent data for resource managers and policy makers[16,17], active acoustic surveys have been used to estimate abundance, biomass, and length and spatial distributions of fish stocks at FSAs[13,18,19]. However, the cost and complexity of collecting and processing data

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Family	Common Names	No. Species	Total Landings (tons)
Clupeidae	Herrings, Shads, Sardines, Menhadens	198	9,087,812
Gadidae	Cods and Haddocks	24	6,637,665
Carangidae	Jacks and Pompanos	146	4,315,926
Sciaenidae	Croakers, Drums, and Weakfishes	283	1,908,360
Sparidae	Porgies	150	439,887
Epinephelidae, Serranidae	Groupers and Sea Basses	537	343,236
Lutjanidae	Snappers	110	268,976
Sebastidae, Scorpaenidae	Rockfishes, Rockcods, and Thornyheads	132, 216	265,410
Mullidae	Goatfishes	87	220,604
Haemulidae	Grunts	133	121,682
Ophidiidae	Cusk-eels	258	41,353
Labridae	Wrasses	520	23,464
Ictaluridae	Catfishes	51	12,733
Acanthuridae	Surgeonfishes, tangs, unicornfishes	82	9,785
Scaridae	Parrotfishes	100	5,266
Acipenseridae	Sturgeons	25	422
Batrachoididae	Toadfishes	83	339
Total		**3,135**	**23,702,920**

Table 1. Families of commercially important fishes that have been documented to produce sounds and form spawning aggregations. The table presents total global landings (tons) reported in 2013 (FAO) for aggregating, sound producing families of fishes. Families are organized by total landings. Sciaenidae, ranking fourth in contributions to landings, supports global economies and is need of new assessment methods to ensure sustained productivity.

have impeded the widespread application and long-term use of active acoustics for assessments of most aggregate spawning fishes, which are often associated with small-scale fisheries in developing countries[6].

Increased recognition of sound production in over 100 families of marine fishes[20, 21] has prompted an insurgence of passive acoustic methods to non-invasively and efficiently study and monitor populations[22–24]. As many fishes produce species-specific calls associated with courtship and reproduction, recordings of sound production have been used to identify the timing and location of spawning in a number of exploited species, such as Atlantic Cod[18] (*Gadus morhua*), Pollock[25] (*Pollachius pollachius*), Haddock[26] (*Melanogrammus aeglefinus*), Nassau Grouper[27] (*Epinephelus striatus*), and numerous species of croakers[28, 29] (family Sciaenidae). While some progress has been made to use received sound levels as indices of fish presence, behaviors, and relative abundance at FSAs[30–34], difficulties in relating sound production metrics to fish densities have precluded passive acoustic estimations of abundances for use in quantitative population assessments[22, 23, 35].

With an understanding of fish calling rates and sound propagation, it is possible to estimate abundance from call occurrences[22, 35, 36]; however, call rates at FSAs often vary considerably[29, 37, 38] and are difficult to characterize in-situ for fishes. Additionally, fish choruses at some FSAs prevent the isolation of individual calls[29]. A more tractable approach may be to utilize relationships between sound production levels, measured using passive acoustics, with independent measures of fish density estimated with active acoustics[22, 35]. Ultimately, species-specific models could be used to assess and monitor spawning stock densities, abundances, and biomasses using passive acoustic methods during periods with stable rates of sound production[33, 39, 40].

Croakers are an ideal assemblage of fish species to develop and test relationships between sound production and independent measurements of fish density at FSAs, because they produce sounds during spawning activities[41, 42] within FSAs that form in estuaries and coastal habitats[43, 44] (Table 1). Also, croaker fisheries generate tens of billions of US dollars globally each year[45, 46], and among the 166 species of croakers for which information is available on the IUCN Red List, those that are threatened or endangered are aggregate spawners that are also overfished[47, 48]. Therefore, new methods that demonstrate the use of passive acoustic technologies to estimate density and assess spawning populations of croakers will support the development of monitoring programs that aid adaptive management strategies to prevent stock declines, hasten recovery, and support sustainable harvest of other vulnerable, aggregating species that produce sound during spawning (Table 1).

In this paper, we developed active and passive acoustic methods to assess the abundance of Gulf Corvina (*Cynoscion othonopterus*; family Sciaenidae), hereafter Corvina, at its FSA and constructed a model to quantify relationships between sound levels and fish density during spawning. From late February to early June, during the outgoing tide over 2–3 day periods prior to new and full moons[43, 49], Corvina migrate from as far south as the Midriff Islands (Fig. 1) and form a FSA at one location in the estuaries of the Colorado River Delta (1–20 m depth) in the Northern Gulf of California, Mexico[43, 46]. During these brief yet predictable events, the FSA is targeted by a commercial gill net fishery of more than 500 small boats, which harvests several thousand tons of Corvina over the course of a few weeks[46]. Fishers locate the FSA through knowledge of their predictable migrations and by cueing on the courtship sounds produced by male Corvina during spawning (see Supplementary Fig. S1). The fishery is managed by a quota system in which estimates of catch per unit effort (CPUE) are used to estimate stock abundance and set catch levels for the spawning season. Despite high fishing pressure and

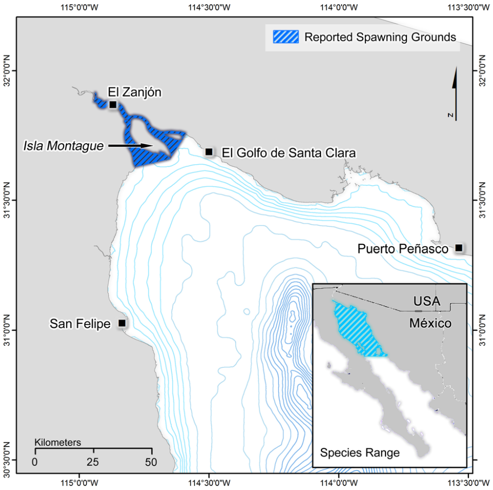

Figure 1. Species range and spawning grounds of Gulf Corvina (*Cynoscion othonopterus*). The species range extends from the Midriff Islands to the northern limit of the Gulf of California (inset map). During the months of February to early June, the reproductive population migrates northward to the only reported spawning grounds in the Colorado River Delta. The map is adapted by permission from Macmillan Publishers Ltd: [SCIENTIFIC REPORTS] (Erisman *et al.*[43]), copyright 2012.

uncertain sustainability[7,43] the stock size and status are largely unknown due to the lack of fisheries-independent data[50]. Therefore, the development of methods to estimate fish density from active acoustic measurements and sound levels would provide accurate estimates of fish abundance and biomass that could inform the quota system by reducing the probability of setting unsustainable harvest limits.

We used active acoustic methods to measure density, lengths, abundance, biomass, and spatial extent of spawning fish and passive acoustic methods to measure the spatial distribution of sound levels attributable to fish sound production across eight surveys in March and April 2014. We then compared independent measures of fish density and sound levels at the FSA site to test the hypothesis that sound levels would increase linearly with density (H_1, Fig. 2), resulting in a relationship with strong predictive power. While the potential for a weaker relationship that becomes asymptotic and insensitive at high densities exists (H_2, Fig. 2), we expected that the careful selection of a sound level metric that is proportional to acoustic power would negate this possibility. We also acknowledged that a predictive relationship may not exist (H_0, Fig. 2) due to the inherent variability in fish sound production rates at FSAs. However, we anticipated that a significant relationship would be observed if a period of stable sound production rate could be identified, resulting in a model to estimate fish density from sound production levels.

Results

Fish distribution, abundance, and biomass.

Over the four days of active acoustic surveys, fish distributions varied between incoming and outgoing tides with higher densities extending progressively farther into the delta on the outgoing tide during spawning (Fig. 3). Differences in the density distributions mapped during the incoming and outgoing tides revealed that the aggregation moved slower than the survey progressed, mitigating the potential to survey individuals more than once. On the incoming tides of 27 March and 27 April 2014 and the outgoing tides of 28 March, 27 April, and 28 April 2014, we surveyed the entire aggregation present in the northeastern channel of the delta as evident from lower densities at the start and endpoints of surveys. Among these days, the aggregation was distributed over a linear distance of 8 to 25 km with densities greater than 5 fish/1000 m^3. On the incoming tides on 28 March and 28 April and outgoing tide on 27 March 2014, the surveys did not sample the entire aggregation due to a miscalculation of the southern extent of the aggregation, absence of large Corvina in the channel, and degraded survey conditions, respectively. We observed large spawning events on all days except for 28 April 2014 when an imminent new moon prompted the aggregation to disperse from the reproductive grounds.

Mean densities (±S.E.) of Corvina ranged from 0.43 ± 0.07 to 15.97 ± 2.04 fish/1000 m^3, which corresponded to an estimated 76,052 ± 12,380 to 1,551,729 ± 177,691 fish (±S.E.) across surveys (Table 2). Mean survey densities were higher on the outgoing tides in comparison to incoming tides, corresponding to the known timing of peak spawning activity, but were not significantly different (one-tailed t-test, $p = 0.073$). We estimated the abundance of Corvina in the northeastern spawning grounds at 1.53 million ± 196,443 and 1.55 million ± 177,691 fish (±S.E.) on the outgoing tides of 28 March and 27 April 2014 when we surveyed the entire aggregation in the

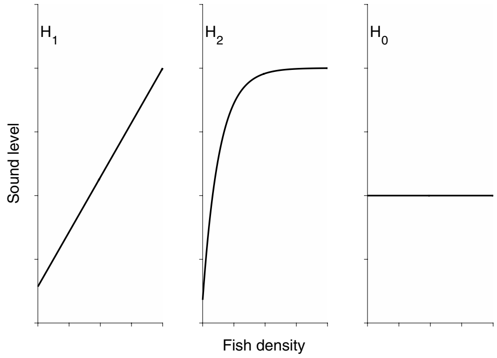

Figure 2. Hypotheses of the relationship between fish sound production levels and density. (H_1) A strong, predictive linear relationship with increases in sound levels as a function of density, (H_2) a weaker non-linear relationship where sound levels fail to respond to high densities, and (H_0) a non-predictive relationship with changes in density not affecting sound levels.

northeastern channel. During the presence of FSAs, we observed fish of total lengths (L) 21–100 cm, as estimated from the following Kirchoff-ray Mode (KRM) model-derived relationship between mean lateral-aspect 120-kHz target strength (TS) and Corvina L (cm):

$$TS = 22.44 \log L - 76.21; \ r^2 = 0.97 \quad (1)$$

The results of the KRM model are detailed in Supplementary Fig. S2. The mean L (±95% C.I.) of the aggregated fish ranged from 38.677 ± 2.761 cm to 53.402 ± 1.407 cm among surveys, corresponding to mean weights (W) of 0.578–1.464 kg (Table 3). Biomass varied across days and tides depending on extent of area surveyed; however, on the outgoing tides of 28 March and 27 April 2014, estimated biomass was $2{,}145 \pm 537$ and $2{,}133 \pm 480$ metric tons (±95% C.I.), respectively (Table 3).

Fish sound production and relationship to density.

We recorded Corvina sound production associated with spawning during all survey days. We did not observe sound production by any other fish species in our recordings. Measurements of mean square pressure amplitude (p^2) over the frequency band of Corvina chorusing (251–498 Hz, see Supplementary Fig. S1) ranged from 0.02 to 12,738 Pa^2 among surveys but varied across the spawning grounds and tidal periods (Fig. 4). Values of mean p^2 per survey were significantly higher on the outgoing tides (one-tailed t-test, $p < 0.001$), coinciding with spawning; mean p^2 (±95% C.I.) ranged from $1.56 \pm 1.9\,\mathrm{Pa}^2$ on the incoming tide of 28 April to $4430 \pm 1004.0\,\mathrm{Pa}^2$ on the outgoing tide of 27 April 2014. Spatial differences in p^2 showed similarities to measured differences in fish density across the spawning grounds during the outgoing tides but not during the incoming tides (Figs 3 and 4). We observed elevated sound levels ($p^2 > 800\,\mathrm{pa}^2$) attributable to dense aggregations and spawning activity over distances of 6.5 to 25 km on the outgoing tides of 27 and 28 March, and 27 and 28 April 2014.

Comparisons between p^2 and densities (ρ, fish/1000 m^{-3}) resulted in different relationships dependent on the timing of measurements in relation to high tide (Fig. 5, ANCOVA, $p < 0.001$). The slopes of hourly regressions from 3 hours before high tide to high tide and from 2 to 5 hours after high tide were not significantly different (multiple comparisons, Tukey-Kramer, $p > 0.52$); however, these time periods were unsuitable to construct a model between p^2 and ρ due to the lack of good line fits and decoupling of changes in sound levels with density. From high tide until two hours after high tide, the slopes of regressions were homogeneous (multiple comparisons, Tukey-Kramer, $p > 0.99$) and significantly different from all other hours (multiple comparisons, Tukey-Kramer, $p < 0.001$), indicative of stationary call rates of Corvina that are additive in p^2 as a function of density during the two-hour window (Fig. 6). The modeled relationship between ρ and p^2 from high tide to two hours after high tide (Fig. 6) resulted in a method to estimate fish density from measured sound production ($F_{1,68} = 216$, $p < 0.001$) with the following equation:

$$\rho = 0.0028p^2 + 2.89; \ r^2 = 0.76 \quad (2)$$

This result did not refute our hypothesis that a relationship exists for a period of stable sound production (H_1, Fig. 2). Equation (2) opens the possibility to estimate ρ, a fundamental measurement for population assessments, across different spatial scales (see Supplementary Figs S3 and S4) and thus abundance and biomass from future measurements of p^2 during the two hours after high tide.

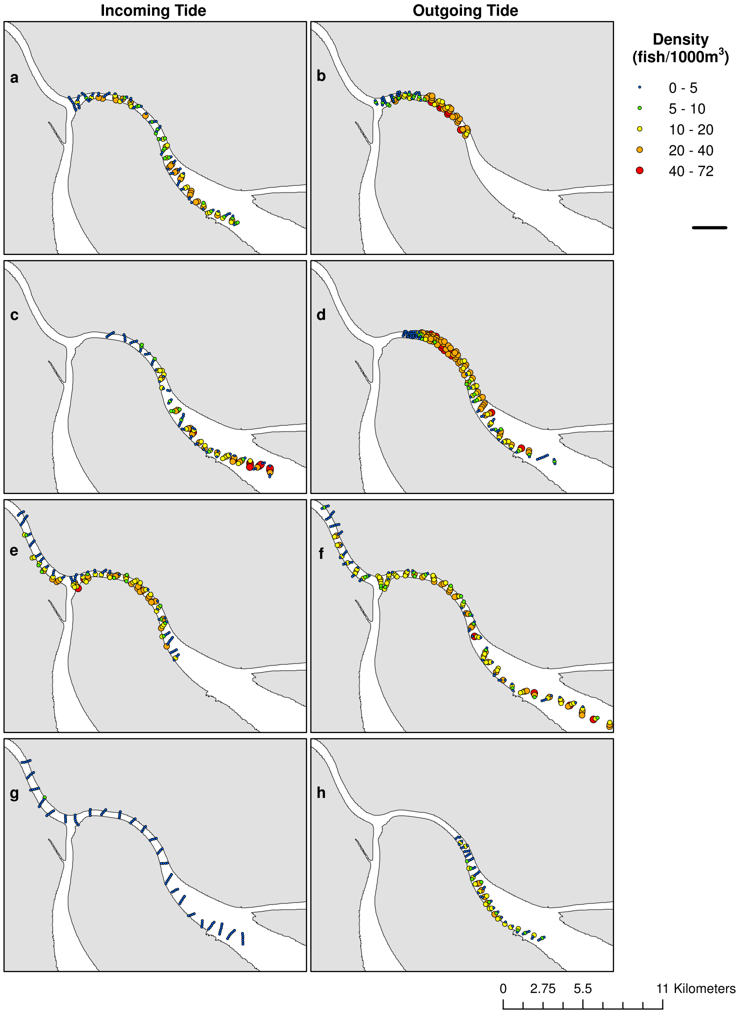

Figure 3. Spatial distribution of Gulf Corvina (*Cynoscion othonopterus*) densities (fish/1000 m^3). Mean fish densities per every 150-m survey length in the spawning grounds of the northeastern channel of the Colorado River Delta on the incoming and outgoing tides on (**a**,**b**) 27 March 2014, (**c**,**d**) 28 March 2014, (**e**,**f**) 27 April 2014, and (**g**,**h**) 28 April 2014. All survey data are depicted, including 150-m survey lengths with densities of 0 fish/1000 m^3. Collection of active acoustic data on the outgoing tide of 27 March 2014 (**b**) ended prior to the termination of passive acoustic data collection due to deteriorated survey conditions. Maps were generated using the ArcMap extension of ArcGIS version 10.2.2 (http://www.esri.com/, ESRI, USA).

Discussion

The results of this study demonstrate that sound levels may be used to estimate the densities of soniferous fishes at FSAs, which provides an alternative means to assess their abundance, biomass, and spatial distribution. The selection of p^2 as an additive metric of Corvina sound production coupled with the identification of a two-hour period of stable sound production as a function of density resulted in the generation of a predictive relationship. While several methods are currently available to provide independent measurements fish density[12] for comparison to sound levels, here we corroborate and further develop the use of active acoustic data to not only provide measures of density in a challenging shallow water environment but also to estimate abundance, mean length, biomass, and spatial distribution of fish. Our results support the continued use of passive acoustic methods to determine

Tide	Volume (m^3)	Density (fish/1000 m^3)	S.E. (fish/1000 m^3)	C.V. (%)	95% C.I. (fish/1000 m^3)	Abundance (fish)	S.E. (fish)	95% C.I. (fish)
27 March 2014								
Incoming	91,966,129	7.47	0.99	13.3	5.86–9.95	686,987	91,046	538,922–915,063
Outgoing	49,576,934	13.04	1.87	14.3	9.32–16.66	646,483	97,709	462,057–825,952
28 March 2014								
Incoming	111,125,728	11.73	1.86	15.9	8.29–15.62	1,303,505	206,694	921,232–1,735,784
Outgoing	96,295,547	15.97	2.04	12.8	12.13–20.16	1,537,840	196,443	1,168,065–1,941,318
27 April 2014								
Incoming	132,894,113	11.00	1.21	11.1	8.60–13.37	1,461,835	160,802	1,142,889–1,776,794
Outgoing	153,181,587	10.13	1.16	11.5	8.18–12.98	1,551,729	177,691	1,253,025–1,988,297
28 April 2014								
Incoming	176,863,975	0.43	0.07	15.3	0.32–0.59	76,052	12,380	56,596–104,350
Outgoing	64,708,929	8.07	1.11	13.8	5.99–10.41	522,201	71,827	387,606–673,620

Table 2. Estimates of density and abundance of mature Gulf Corvina (*Cyonscion othonopterus*) per survey. S.E. = standard error; C.V. = coefficient of variation (%); 95% C.I. = 95% confidence interval.

Tide	Length (cm)	S.E. (cm)	Weight (kg)	Biomass (kg)	S.E. (kg)	95% C.I. (kg)
27 March 2014						
Incoming	53.112	0.520	1.441	989,948	131,555	732,100–1,247,797
Outgoing	52.846	0.525	1.421	918,652	132,055	659,824–1,177,480
28 March 2014						
Incoming	51.442	0.568	1.315	1,714,109	272,461	1,180,086–2,248,132
Outgoing	52.512	0.338	1.395	2,145,287	274,386	1,607,491–2,683,082
27 April 2014						
Incoming	52.798	0.407	1.417	2,071,420	228,415	1,623,727–2,519,114
Outgoing	52.249	0.447	1.375	2,133,627	245,006	1,653,417–2,613,838
28 April 2014						
Incoming	38.677	1.409	0.578	43,958	7,333	29,586–58,331
Outgoing	53.402	0.718	1.464	764,502	105,656	557,417–971,588

Table 3. Mean total length, weight, and estimated biomass of mature Gulf Corvina (*Cynoscion othonopterus*) per survey. S.E. = standard error; 95% C.I. = 95% confidence interval. Mean total length on the incoming tide of 28 April 2014 reflects the absence of high abundances of large Gulf Corvina within the survey area.

the spatio-temporal distributions of spawning activity and relative abundance[27, 29, 31, 34, 37]. More importantly, they show that when these methods are calibrated to the spatial and temporal dynamics of spawning activity of the study system, the difficulties of relating sound levels to actual fish densities can be overcome to provide a new fisheries-independent method to assess FSAs.

The selection and validity of different methods to estimate density for comparison to passive acoustic measurements are often challenged and limited by resource and equipment availability, environmental conditions, and spatial and reproductive dynamics of target species[12]; thus, different FSAs pose unique challenges that need to be considered. For example, aspects of the Corvina FSA present a series of obstacles that have thus far impeded fisheries-independent assessments: the aggregation forms in a remote shallow water environment (1–20 m) with near-zero visibility and large tidal ranges (7 m over a 6 hour period), which inhibits visual surveys; spawning takes place within a no-take biosphere reserve thereby discouraging invasive trawl sampling; and heavy fishing mortality during stock migrations and some spawning events within the protected reserve conflicts with mark-recapture, telemetry, and extractive methodologies. In this study, the versatility of active acoustic methods overcame these challenges to elucidate spawning and stock dynamics of Corvina and produce measurements of fish density, abundance, and biomass in a non-invasive manner.

Our active acoustic surveys generated the first fisheries-independent estimate of Corvina density, abundance, mean length, biomass, and spatial distribution of mature fish across four days of spawning in 2014 that may supplement existing catch per unit effort data to develop more robust and comprehensive stock assessments for the fishery. Although the active acoustic survey results elucidated the dynamics of the aggregation and the fishery, they were difficult to conduct due to sound propagation in shallow water environments, low insonified volumes, and potential vessel avoidance by fish[51, 52]. These challenges were mitigated by orienting the acoustic beam axis nominally 10° below horizontal to detect Corvina up to 50 m away from the vessel and removing the first 10 m range of acoustic data that exhibited low detection probability, respectively. As opposed to echo-integration[16], fish were detected as tracks with multiple, spatially coherent *TS* measurements, permitting the calculation of density, abundance, and *TS* distributions of aggregated Corvina. The selection of Corvina-specific detection parameters and a lack of bycatch in the fishery[50] provide credence that our estimates were not influenced by the presence

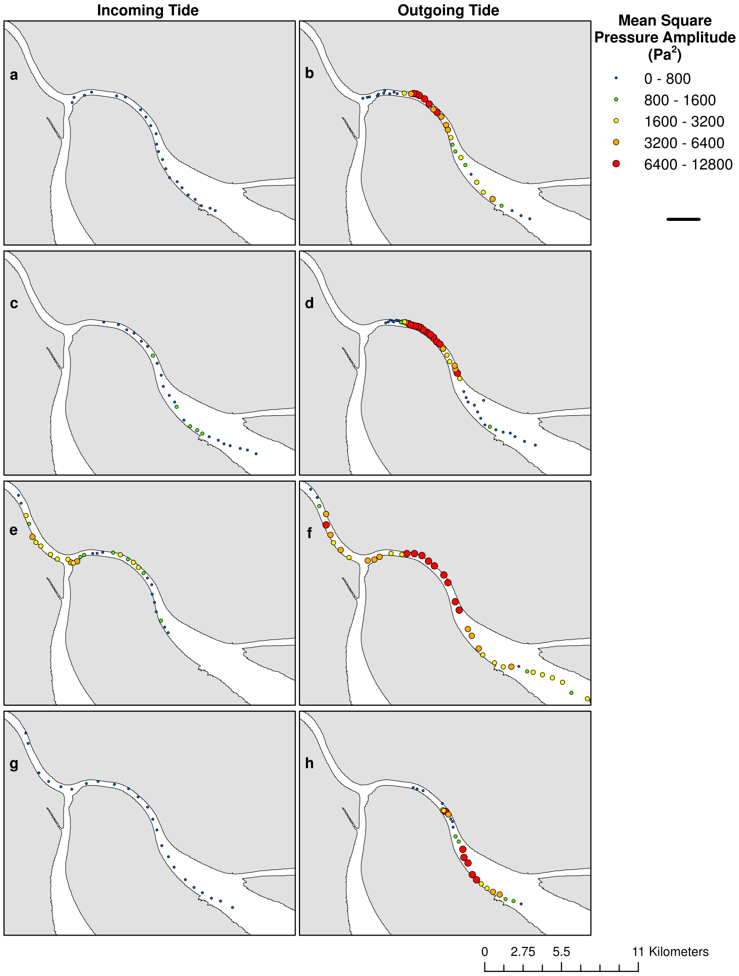

Figure 4. Spatial distribution of mean square pressure amplitude (p^2; Pa^2; 251 Hz–498 Hz). Sound levels over the frequency band of Gulf Corvina (*Cynoscion* othonopterus) chorusing in the spawning grounds of the northeastern channel of the Colorado River Delta on the incoming and outgoing tides on (**a**,**b**) 27 March 2014, (**c**,**d**) 28 March 2014, (**e**,**f**) 27 April 2014, and (**g**,**h**) 28 April 2014. Collection of passive acoustic data on the outgoing tide of 27 March 2014 (**b**) continued after the conclusion of the active acoustic survey. Maps were generated using the ArcMap extension of ArcGIS version 10.2.2 (http://www.esri.com/, ESRI, USA).

of additional species. KRM modeling provided an equation to relate mean lateral-aspect *TS* to fish length for Corvina, thus enabling the estimation of mean fish lengths, weights, and total biomass.

While this study observed the formation of multiple FSAs comprised of over 1 million fish, these data do not implicitly provide an estimate of the total spawning population. To assess the total abundance and biomass of spawning populations using active or passive acoustics, the spatio-temporal dimensions and reproductive dynamics of FSAs need to be known. For example, previous efforts that have tracked fishing intensity at the FSA have shown that the Corvina likely aggregate in both channels of the Colorado River Delta[43]. As we only focused our efforts in the northeastern channel due to logistical limitations, only a portion of the spawning grounds were sampled. Additionally, FSAs of Corvina form prior to the new and full moons in the months of February to June, but it remains unknown whether the entire reproductive population participates in all spawning events with fixed residency times, if individuals spawn only once or multiple times, if mean length and weight across all spawning

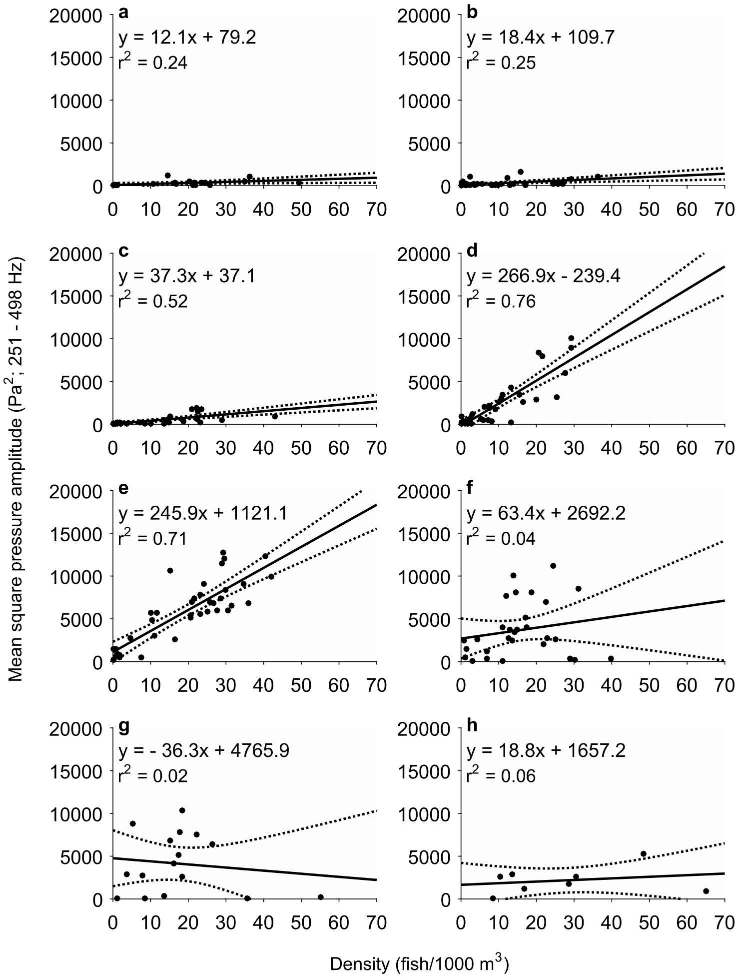

Figure 5. Relationship between sound levels and fish density over one-hour periods. Regressions of mean square pressure amplitude (p^2; Pa^2; 251–498 Hz) versus fish density (ρ; fish/1000 m^3) from (**a**) 3–2 hours before high tide, (**b**) 2–1 hours before high tide, (**c**) 1–0 hours before high tide, (**d**) 0–1 hours after high tide, (**e**) 1–2 hours after high tide, (**f**) 2–3 hours after high tide, (**g**) 3–4 hours after high tide, and (**h**) 4–5 hours after high tide. Dotted lines indicate 95% confidence intervals. Densities are mean densities per 150-m survey length that were nearest in space and time to sound measurements. See Supplementary Fig. S3 for hourly relationships using mean density per 300-m survey length.

events are constant, and what are the effects of fishing mortality during migrations to the delta. However, with the future documentation of these dynamics, active and passive acoustic estimates can be calibrated using a hierarchical model to determine the total abundance and biomass of the spawning population[53].

Fish sound production rates at FSAs are difficult to measure and highly variable, often influenced by the timing of spawning activity[29, 37, 38], resulting in temporally inconsistent relationships between sound levels and abundance, as observed in this study. Consequently, the use of call numbers as predictors of fish abundance[35, 36] has proven challenging for researchers[22, 23]. For many species, sound production associated with courtship and spawning is only present in males[26, 41], thus without a knowledge of sex ratios, the use of call rates and occurrences to estimate abundance and density may only be applicable to males, assuming factors affecting sound production rates are understood. However, it has been argued that the call rates of courting males may be influenced by the

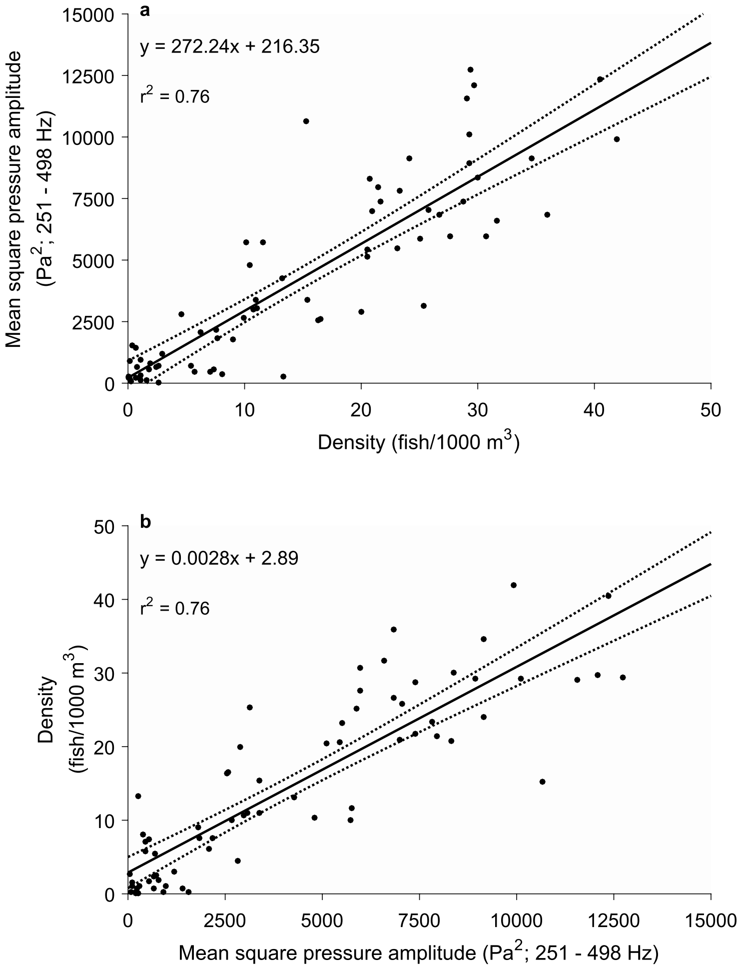

Figure 6. Relationship between sound levels and fish density during the peak spawning period. (**a**) Mean square pressure amplitude (p^2; Pa^2; 251–498 Hz) as a function of density (ρ; fish/1000 m^3) for measurements during the first two hours after high tide and (**b**) the modeled relationship generated for estimating ρ from future measurements of p^2, ($F_{1,68} = 216$, $p < 0.001$). Dotted lines indicate 95% confidence intervals. Densities are mean densities per 150-m survey length that were nearest in space and time to sound measurements. See Supplementary Fig. S4 for the modeled relationships using mean density per 300-m survey length.

presence of females, where call levels may be indicative of both male and female density[33] but these relationships have not been fully investigated. At some FSAs high abundances of fish and sound production prevent the detection of individual calls altogether, as in this study, requiring measurements of ambient sound pressure levels[31, 54] and the designation of indices to infer aggregation sizes[30, 32].

Given these challenges and uncertainties, we compared fish sound production to independent measurements of density, which may be a more feasible manner to model their relationship at FSAs[22, 35]. Previous works have exemplified the potential of this method through comparisons of sound production indices with CPUE of simultaneous trawls[39], densities of early stage eggs[31], and asynchronous fish densities estimated with active acoustics[40] and visual census[33]. In this study, we compared p^2 over the bandwidth of Corvina chorusing to fish density estimated simultaneously with active acoustics. We selected p^2 over other measurements, such as root-mean-square sound pressure level (dB), because the summed power from incoherent sounds from multiple calling fish is proportional to p^2. By comparing the relationship of p^2 with density over several one-hour periods, we were able to model the time-evolving relationship and identify patterns of similarity. The insensitivity of sound levels as a function of density prior to high tide and beyond two hours after high tide suggested inconsistencies in fish chorusing rates and a lack of coordinated courtship behaviors outside the peak spawning period. From high tide to two hours after high tide we observed a stable positive relationship, where increases in density corresponded linearly to increases in p^2, indicating a two-hour period of homogeneous sound production rates during which density can be estimated from passive acoustic measurements. Importantly, comparisons of regression slopes provided an approach to identify periods of stationary sound production, thereby negating the need to estimate calling and chorusing rates of males and sex ratios in a challenging environment. However, this strategy assumed that sex ratios remained stable across measurements, which has been estimated at 1:1 from fisheries landings[49].

As with most assessment methods, the application of the model developed in this study for population monitoring and fishery management is limited by the inherent uncertainty of density estimates from sound levels, warranting confidence intervals and the potential for future improvement with additional data and possible sources of variance, such as tidal current strength. However, this model presents an opportunity to assess Corvina density and calculate abundance with confidence intervals, during the two hours after high tide, using passive acoustic survey techniques. With this relationship, sound production measurements can be used to monitor the spatio-temporal distribution of density and abundance and quickly detect potential impacts of fishing activities on stock size and spawning activity. Estimates of abundance can also be used to calculate biomass if fish lengths at the FSA site are known through independent sampling. Lengths may also be estimated using passive acoustics through analyses of the fundamental frequencies of sounds[55].

Our results support the application of both active and passive acoustics to conduct surveys of FSAs that could provide needed information to resource managers, policymakers, conservationists, and fishing communities. We highlight the growing potential to develop comparative models between sound production and density of fishes using accurate independent measurements of fish density, but we acknowledge the future importance of testing their robustness against more complex acoustic modeling approaches with additional covariates that affect sound propagation, such as water depth, bathymetry, and boundary conditions[35]. Although our methods were applied to Corvina, they may be further developed and adapted to other commercially important species of soniferous fishes that form FSAs (Table 1), such as members of the cod, grouper, and croaker families[25,29,34], based on knowledge of study species and systems and the appropriate spatial and temporal scales for predicting abundance from sound levels[35]. Active acoustic surveys can be designed to provide estimates of density, abundance, and biomass at FSAs; however, other independent sources of density may be viable, such as visual census, if conditions permit. The inclusion of fixed, long-term acoustic recorders in future efforts at FSAs will enable entire spawning grounds to be assessed simultaneously over multiple spawning events, providing a cost effective means to capture ambient sound and estimate abundance across a suite of habitats and economic environments. The continuation, improvement, and expansion of these methods across multiple species will validate passive acoustics as a frontier tool for the management and conservation of fish populations.

Methods

All methods were conducted in accordance with approved guidelines and regulations. The use of deceased fish from the fishery for *TS* modeling and collection of acoustic data were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California San Diego under IACUC protocol S13240 and at the University of Texas at Austin under IACUC protocol AUP-2015-00162.

Survey design. Prior to the new moons in March and April 2014, we simultaneously conducted eight active and passive acoustic surveys in the northeastern channel of the Colorado River Delta from two small (8 m long) fishing vessels. As there are only two days of peak spawning per FSA event (2–3 days before new and full moons)[43], we focused our efforts on these days to assess the aggregation and minimize daily variation. We surveyed the spawning grounds of Corvina twice daily, on the incoming and outgoing tide, progressing in the direction of tidal current (see Supplementary Fig. S5 and Table S1). Active acoustic surveys of Corvina were comprised of semi-randomized parallel transects across the delta channel spaced, on average, 0.5 km apart with deviations attributable to tidal flow and avoidance of surface gill nets. From a second vessel, we conducted passive acoustic surveys of Corvina sound production in synchrony with the timing and location of each active acoustic transect (see Supplementary Fig. S5), and the independent measurements were compared to estimate the spatial distribution of sound levels attributable to spawning Corvina and to model the relationship between acoustic measurements of density and sound production.

Active acoustic sampling. We conducted active acoustic sampling with a 120-kHz echosounder (ES60, Simrad-Kongsberg, Norway) configured with a split-beam transducer (ES120-F; Simrad-Kongsberg, Norway) having a nominal 9° beam width. Prior to sampling, we calibrated the complete system using the standard sphere method[56,57] and a 38.1 mm diameter sphere constructed of tungsten carbide with 6% cobalt binder material (Bal-tec™, Micro Surface Engineering, Inc., USA). For each survey, we collected water temperature and salinity profiles using a handheld CTD (Castaway®-CTD, SonTek/Xylem, Inc., USA) to measure sound speeds, calculate absorption coefficients, and calibrate data during processing. Throughout the surveys, the echosounder operated 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 positioned off the port side of the vessel with the face nominally 0.53 m below the sea surface. As the survey area ranged in depth from 1 to 20 m, the beam axis was oriented nominally 10° below horizontal to increase the insonified volume, limit reverberation from the water surface, and increase the observational range. Surveys were performed at an average speed of 6 knots, and received power and split-beam phase data, indexed by time and geographic location, were sampled and stored every 64 µs.

Passive acoustic sampling. We recorded ambient sound at a single random position along each active acoustic transect (see Supplementary Fig. S5), 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). The location of a recording along each transect was dictated by ability of the vessel to position itself along the next transect at the same time as the active acoustic vessel passed by. Files were digitized with 24-bit resolution at a sampling rate of 192 kHz and stored (.wav format) on a secure digital high capacity (SDHC) memory card. At each sampling location, the vessel engine was turned off, and 60 s of ambient sound was recorded with the hydrophone lowered 2 m below the hull of the vessel as the vessel drifted across the path of the portion of the active acoustic transect recently completed.

Specimen collection and target strength modeling. To identify Corvina in the active acoustic dataset and estimate mean L and W, we collected five individuals from the local fishery and used measurements of their shapes and L values (27.4–75.3 cm) to parameterize the Kirchoff-ray Mode model[58] (KRM) and estimate their target strength (TS) values at 120 kHz. The range of L approximated the size range ($L = [21, 100\,\text{cm}]$) of mature Corvina at the FSA[46, 49, 50]. Each specimen was photographed, weighed, measured, and stored on ice before radiography. We radiographed fish ventrally and laterally at a distance of 116 mm with 48 kV at 15 mAs using a MinXray HF100+ mobile X-ray unit (MinXray, Inc., USA). Fish bodies and swim bladders were traced from X-ray images and used to parameterize the KRM. We estimated the average lateral-aspect TS for each length by summing the backscattering cross sections across incident angles of 65° to 115° (90° equals broadside), dividing by 180°, then converting to decibels[58]. The relationship between TS at 120 kHz and Corvina L (cm) was determined by a nonlinear least squares fit of the following equation:

$$TS = m \log L + b$$

Active acoustic data analysis. Active acoustic data were calibrated, visualized, and processed using commercial software (Echoview V5.4, Echoview Software Pty Ltd, Australia). We identified seabed echoes using an automatic detection algorithm and manual editing, and excluded them from further analyses. Echoes within the near-field range (0.51 m), corresponding to a beam-axis depth of 0.62 m, were also removed before further processing. We visually examined echograms to identify and remove regions of noise, propeller wash, bubbles, and along-shore segments of the surveys, resulting in a series of noise reduced across-channel transects for each survey. We identified individual targets attributable to Corvina using a single target detection operator (Split Beam Method 2, Echoview Software Pty Ltd, Australia) and TS and angular-position operands. A conservative, minimum-TS threshold of −46.5 dB was chosen, based on the mean KRM-TS versus L (see Supplementary Fig. S2), to include measures of all sizes of mature individuals potentially within the spawning aggregation[49, 50]. We refined the parameters of single target detection after the completion of sensitivity analyses (see Supplementary Table S2). To identify individual fish from multiple single targets, fish tracks were detected using a tracking algorithm (Alpha-Beta, Echoview Software Pty Ltd, Australia) parameterized with limits on range, alongships- and athwartships-angles, and time. We required a minimum of two single targets to generate a track and adjusted additional parameters for proficiency based on vessel speed and transducer orientation (see Supplementary Table S2).

We gridded fish track detections into 1-m range bins and exported with summed wedge volume[59] (m^3) per bin to estimate the probability density function (PDF) of fish density versus distance from the transducer face and depth. Regions of data corresponding to ranges less than 10 m (2.3 m depth) from the transducer were excluded from further analyses, as a result of exhibiting a non-stationary density PDF attributed to low beam volume, fish avoidance of the vessel, or both[16, 52]; beyond 10 m range (2.3 m depth) fish densities were stationary and homogenous. We separately partitioned the remaining data (range 10 m to the seabed) into 150-m and 300-m survey lengths and complete across-channel transects and exported them with summed wedge volume, total number of fish tracks, and mean geographic position and time per partition. We estimated fish density (fish/1000 m^3) for each 150-m and 300-m survey length and complete across-channel transect by dividing the total number of fish tracks by summed wedge volume. The spatial distributions of fish densities were visualized in geographic information system software (ArcMap, Esri, USA).

Mean fish density per survey was calculated by a transect-volume weighted average of across-channel transect densities. Standard errors, coefficients of variation, and 95% confidence intervals (95% C.I.) of survey densities were estimated from bootstrap resampling ($n = 10{,}000$) of mean density of across-channel transect densities[16, 17]. We estimated fish abundance per survey by multiplying mean fish density by total survey volume as determined from bathymetric data and time-evolving tidal height. Standard errors and 95% C.I. of abundance were estimated by multiplying the bootstrap-estimated values for density by the volume of surveys. Autocorrelation analysis was conducted to ensure statistical independence between across-channel transect densities and the unbiased estimation of variance[17]. However, for systematic surveys with strong spatial trends in fish densities (e.g., aggregated or correlated), variance can be biased high, potentially warranting post-stratification of data to mitigate variance inflation[17, 60]; this potential bias was not evaluated in this study. We tested that mean density on incoming tides was lower than outgoing tides with a one tailed t-test ($\alpha = 0.05$) after the data were tested for homoscedasticity with Levene's test ($\alpha = 0.05$) and normality with an Anderson-Darling test ($\alpha = 0.05$).

To estimate mean fish length and biomass for each survey, we isolated mean TS of fish tracks with at least three consecutive measures to increase the probability of detections with multiple incidence angles. We converted mean TS of fish tracks to L using the derived KRM-TS versus L equation, and mean L per survey was calculated. We converted mean L to mean weight using a length-to-weight relationship previously estimated for Corvina[49, 50] and multiplied by total abundance to estimate spawning stock biomass per survey. Variances of mean L and abundance were propagated and summed in quadrature as fractional uncertainties to estimate the standard errors and 95% C.I. of biomass.

Passive acoustic data analysis. We visually and audibly inspected recordings of ambient sound and extracted 20-s segments free of nearby boat noise and operational disturbances. For each 20-s recording, the mean square pressure amplitude (p^2; Pa^2) over the 251–498 Hz band was measured by integrating the pressure spectral density (μPa^2/Hz; Hanning window; 16384-point FFT) across the peak frequency bandwidth of Corvina chorusing (see Supplementary Fig. S1), thereby limiting contributions from nearby vessels. We plotted p^2 at each sampling location in geographic information system software (ArcMap, Esri, USA) to visualize the spatial and temporal distribution of sound production and their relation to fish densities. We tested that mean p^2 on the incoming tide was lower than on outgoing tides with one-tailed t-test ($\alpha = 0.05$) after the data were tested for homoscedasticity with Levene's test ($\alpha = 0.05$) and normality with an Anderson-Darling test ($\alpha = 0.05$).

Comparison of active and passive acoustic data. Fish densities per 150-m and 300-m survey length (ρ; fish/1000 m^3) that were nearest in space and time to each passive acoustic recording station were compared to p^2 measurements separately. Measurements of ρ and p^2 that were not coupled in space and time were excluded from comparisons. We generated and examined plots of p^2 vs. ρ as a function of time in relation to high tide to test our hypotheses that a relationship with either strong (H_1, Fig. 2) or weak (H_2, Fig. 2) predictive power exists among measurements. We compared p^2 and ρ over one hour periods (3 hrs before high tide – 5 hrs after high tide) to observe the evolving relationship between sound production and fish density. We generated regressions between p^2 vs. ρ for each hour using generalized linear model (GLM) regressions. We compared regression slopes using analysis of covariance (ANCOVA, $\alpha = 0.05$) to test for homogeneity and through multiple comparisons (Tukey-Kramer Method, $\alpha = 0.05$) to identify the time period with a stable relationship between p^2 and ρ. We combined hour periods with homogeneous regression slopes to test our hypotheses and construct a model (GLM) to estimate ρ from p^2. Results from complementary analyses using densities over 150-m and 300-m survey lengths were compared and tested for homogeneity using analysis of covariance (ANCOVA, $\alpha = 0.05$) and found to not be significantly different; thus, comparative results are only presented for modeled relationships between p^2 and ρ per 150-m survey length (see Supplementary Figs S3 and S4 for a detailed description of 300-m results).

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Acknowledgements

We are grateful for the assistance of C. López-Sagástegui, S. E. Freeman, J. Montañez Rivera, B. Legare, A. Montes Zamudio, J. Cabrera Rivera, E. Cabrera Rivera, Y. Flores, and M. Moreno-Báez for their efforts in helping with fieldwork, data collection, and project logistics. We are indebted to the Comisión Nacional de Acuacultura y Pesca (CONAPESCA) and the Comisión Nacional de Áreas Naturales Protegidas (CONANP) for their willingness to support and provide us with the necessary permits to conduct research inside the Upper Gulf of California and Colorado River Delta Biosphere Reserve. Additionally, we would like to express our gratitude to T. S. Sessions, J. P. Zwolinski, J. S. Renfree, and S. A. Mau at the NOAA/National Marine Fisheries Service and the Southwest Fisheries Science Center and G. L. D'Spain, B. X. Semmens, P. A. Hastings, J. B. Shurin, G. B. Deane, and M. J. Buckingham at the University of California San Diego and Scripps Institution of Oceanography for their intellectual support. The project was funded in part by grants to J.R.H. and B.E.E from the NOAA Advanced Acoustics Program and grants to O.A.O. and B.E.E. from the Walton Family Foundation and the David and Lucile Packard Foundation.

Author Contributions

T.J.R., B.E.E., and D.A.D. designed the study, performed analyses, and wrote the manuscript; T.J.R., B.E.E., D.A.D., and J.J.C. collected data; B.E.E., D.A.D., O.A., and J.R.H. supervised the project and assisted in analytical methods. All authors reviewed and edited the manuscript.

Additional Information

Supplementary information accompanies this paper at doi:10.1038/s41598-017-03383-8

Competing Interests: The authors declare that they have no competing interests.

APPENDICES

Supplementary Information

Estimating fish abundance at spawning aggregations from courtship sound levels

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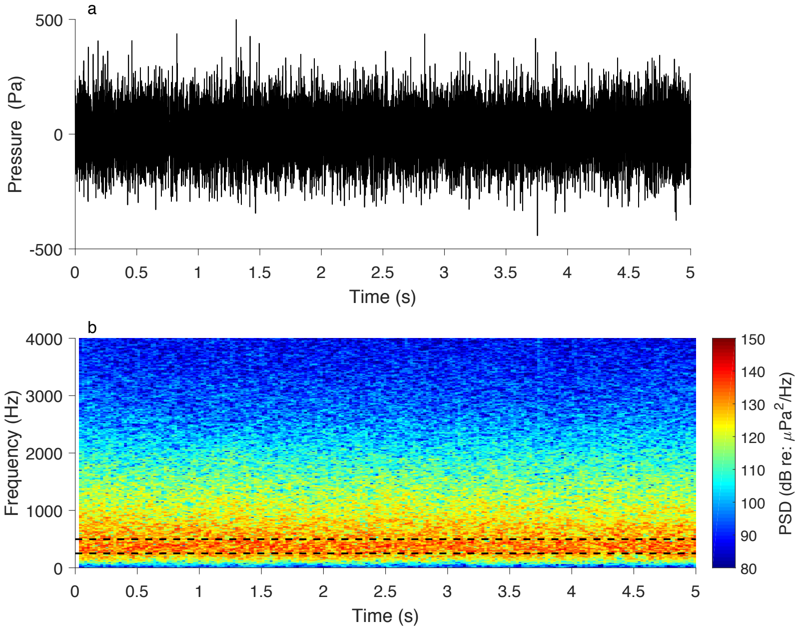
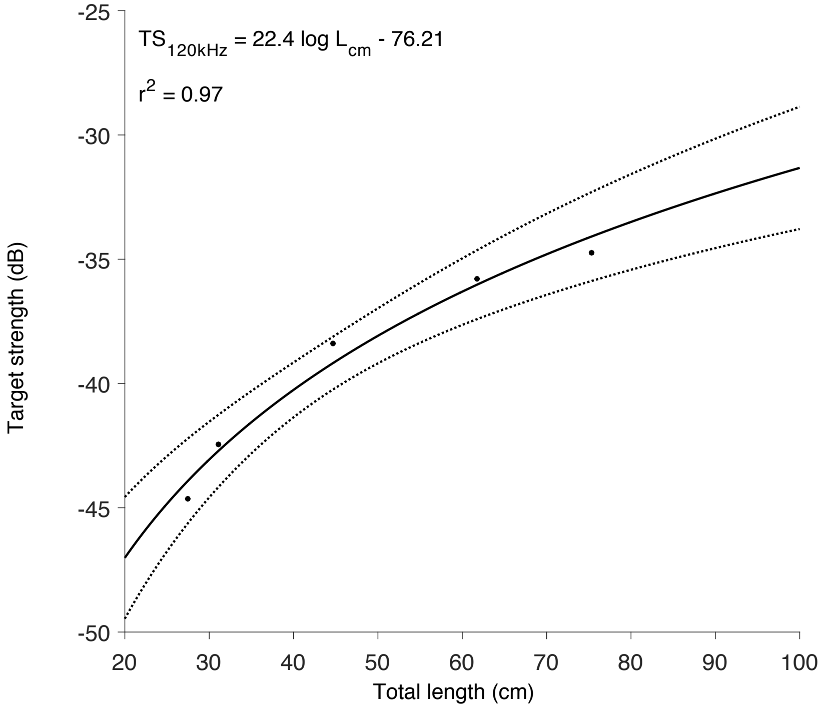
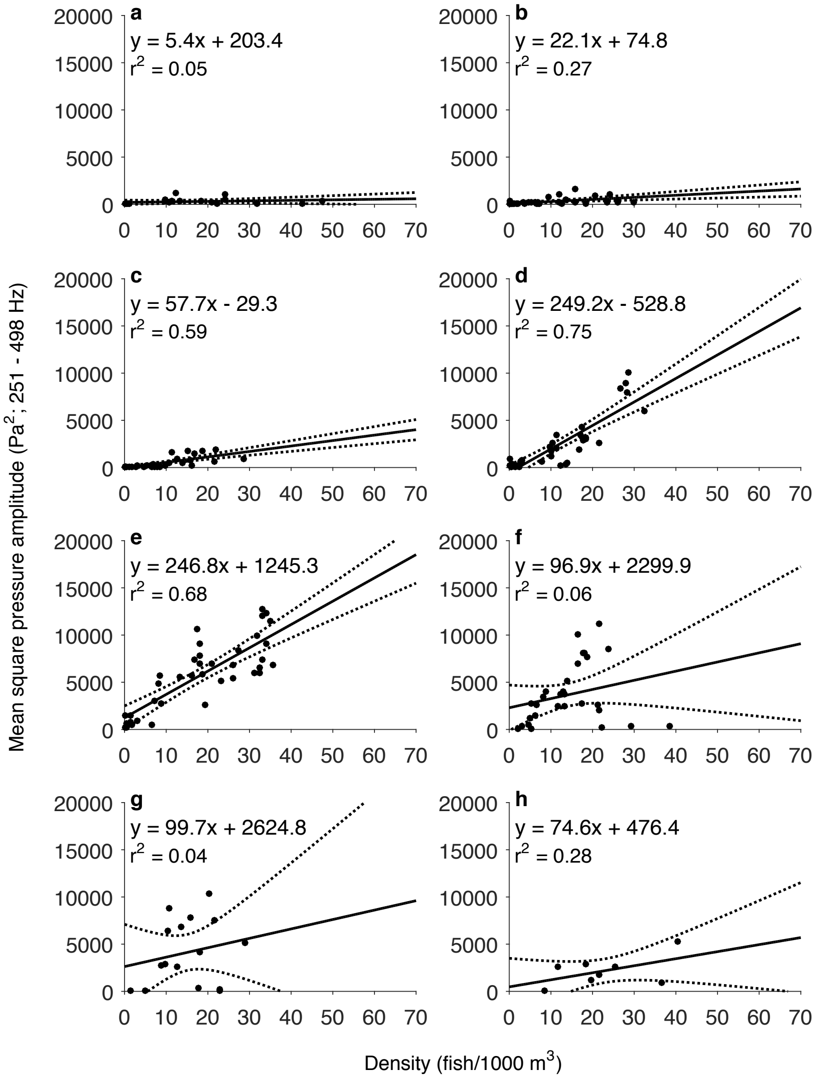
Supplementary Figures



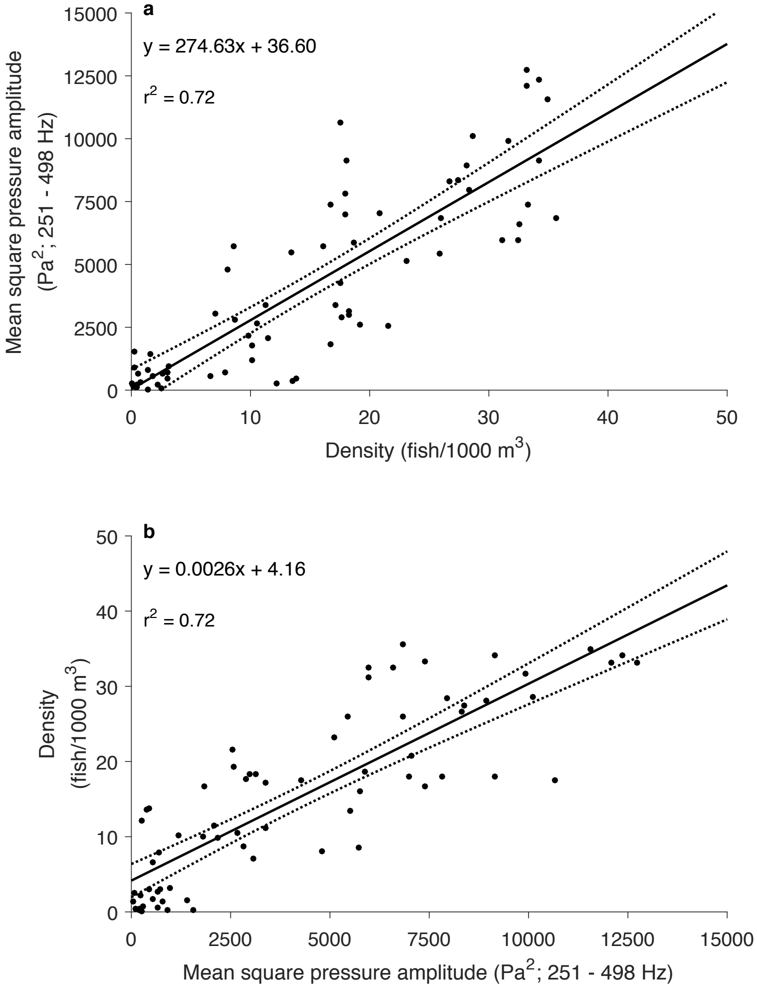
Supplementary Figure S1. Oscillogram and spectrogram of a period of sustained Gulf Corvina (*Cynoscion othonopterus*) chorusing. (a) An oscillogram of a 5-s period of ambient sound recorded at the spawning grounds in the Colorado River Delta, depicting the high amplitudes (Pa) of sound produced by chorusing. (b) A spectrogram of ambient sound over the same 5-s period. The frequency bandwidth of Gulf Corvina chorusing is evident by higher amplitudes of PSD (pressure spectral density). The bandwidth (251 – 498 Hz) over which mean square pressure amplitude (p^2) was integrated is indicated by the dashed black line.



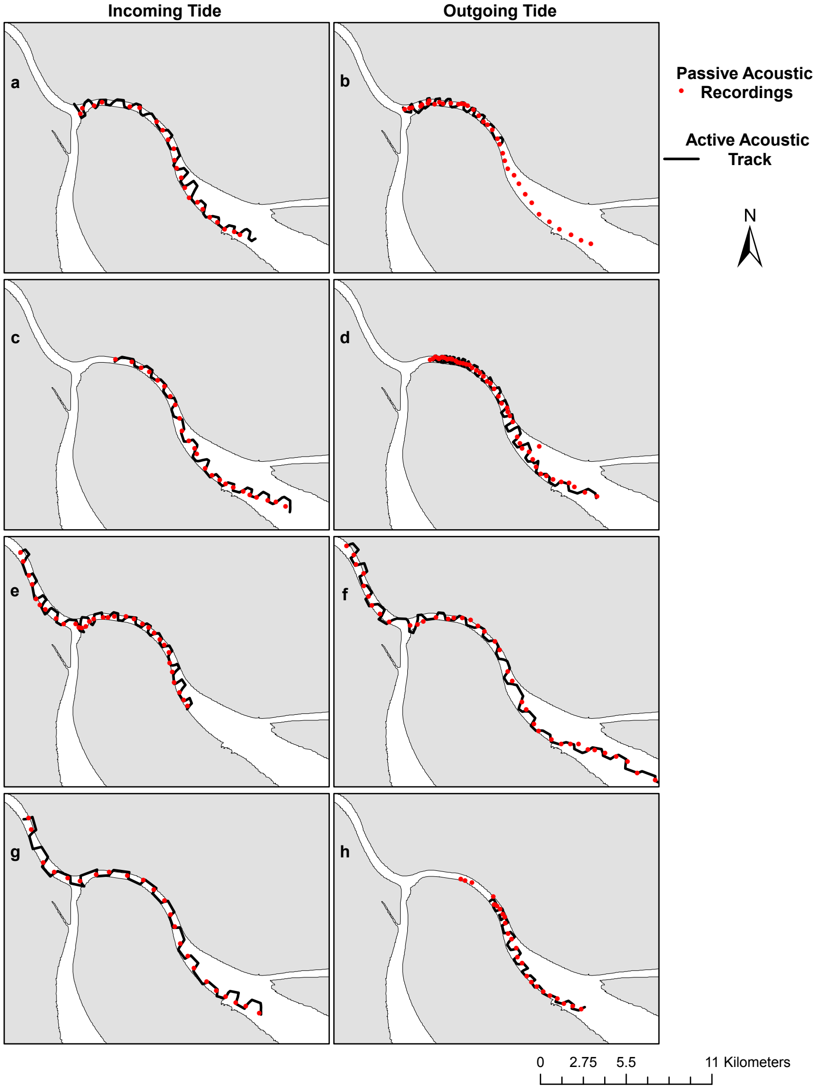
Supplementary Figure S2. Kirchoff-ray Mode (KRM) model-derived relationship between mean lateral-aspect 120-kHz target strength (*TS*) and Gulf Corvina (*Cynoscion othonopterus*) total length (*L*; cm). The model was fitted using a range of *L* of Corvina known to be present at the fish spawning aggregation site. The resulting model parameters are comparable to the reported values for other physoclistous fishes, see *Simmonds J. & MacLennan D. Fisheries Acoustics: Theory and Practice (eds. Simmonds, J. & MacLennan, D.) 1-437 (Blackwell Science Ltd, 2005)*. While the relationship and estimation of variance may be improved with additional data, the model was concluded to be adequate as a tertiary result used within the methodology due to the alignment of parameter estimates with reported values coupled with a high coefficient of determination ($r^2 = 0.97$). The model was used to select a conservative threshold of *TS* for single target detection and estimate the mean *L* of Corvina in survey areas. Dotted lines indicate 95% confidence intervals.



Supplementary Figure S3. Relationship between sound levels and fish density per 300-m survey length over one-hour periods. Regressions of mean square pressure amplitude (p^2; Pa^2; 251 – 498 Hz) versus fish density (ρ; fish/1000 m^3) from (a) 3 – 2 hours before high tide, (b) 2 – 1 hours before high tide, (c) 1 – 0 hours before high tide, (d) 0 – 1 hours after high tide, (e) 1 – 2 hours after high tide, (f) 2 – 3 hours after high tide, (g) 3 – 4 hours after high tide, and (h) 4 – 5 hours after high tide. Dotted lines indicate 95% confidence intervals. Densities are mean densities per 300-m survey length that were nearest in space and time to sound measurements. The slopes of hourly regressions between p^2 and densities per 300-m transect lengths (ρ; fish/1000 m^{-3}) were significantly different (ANCOVA, $p < 0.001$). The slopes of hourly regressions from 3 hours before high tide to high tide and from 2 to 5 hours after high tide were not significantly different (multiple comparisons, Tukey-Kramer, $p > 0.62$); however, these time periods were unsuitable to construct a model between p^2 and ρ due to the lack of good line fits and decoupling of changes in sound levels with density. From high tide until two hours after high tide, the slopes of regressions were homogeneous (multiple comparisons, Tukey-Kramer, $p > 0.99$) and significantly different from all other hours (multiple comparisons, Tukey-Kramer, $p < 0.04$) except for comparisons between 0 – 1 and 3 – 4 hours after high tide (multiple comparison, Tukey-Kramer, $p = 0.35$), 0 – 1 and 4 – 5 hours after high tide (multiple comparison, Tukey-Kramer, $p = 0.15$), 1 – 2 and 3 – 4 hours after high tide (multiple comparison, Tukey-Kramer, $p = 0.42$), and 1 – 2 and 4 – 5 hours after high tide (multiple comparison, Tukey-Kramer, $p = 0.18$).



Supplementary Figure S4. Relationship between sound levels and fish density per 300-m survey length during the peak spawning period. Mean square pressure amplitude (p^2; Pa^2; 251 – 498 Hz) as a function of density (ρ; fish/1000 m^3) for measurements during the first two hours after high tide and (b) the modeled relationship generated for estimating ρ from future measurements of p^2, (GLM; $F_{1,68} = 174$, $p < 0.001$). Dotted lines indicate 95% confidence intervals. Densities are mean densities per 300-m survey length that were nearest in space and time to sound measurements. The slopes of regressions between p^2 and densities per 150-m and per 300-m survey length were not significantly different (ANCOVA, p = 0.93), indicating a stable relationship across two different spatial scales.



Supplementary Figure S5. The locations of passive acoustic recordings and vessel tracks of active acoustic surveys. The two vessels worked in coordination to survey the spawning grounds of the northeastern channel of the Colorado River Delta on the incoming and outgoing tides on (a, b) 27 March 2014, (c, d) 28 March 2014, (e, f) 27 April 2014, and (g, h) 28 April 2014. On average one passive acoustic recording was made per across-channel transect completed by the vessel conducting active acoustic sampling. Passive acoustic recordings were made as the vessel drifted over the tracks of active acoustic transects. Passive and active acoustic measurements that were not coupled in space and time were not included in comparative analyses and model generation. Active acoustic sampling ended prior to the completion of passive acoustic sampling on the outgoing tide of 27 March 2014 (b) due to aberrant survey conditions. Maps were generated using the ArcMap extension of ArcGIS version 10.2.2 (http://www.esri.com/, ESRI, USA).

Supplementary Tables

Table S1. The start and end times of active acoustic surveys. Times are provided as Greenwich Mean Time (GMT) and hours in relation to high tide (re: high tide). Negative values signify hours prior to high tide, while positive values signify hours after high tide. Time at high tide was determined from tidal predictions for El Golfo de Santa Clara, Sonora, Mexico. *Next calendar day (GMT).

Tide	Start Time (GMT)	Start Time (re: high tide)	End Time (GMT)	End Time (re: high tide)
27 March 2014				
Incoming	16:52	-2.4	19:02	-0.23
Outgoing	19:02	-0.23	21:04	1.8
28 March 2014				
Incoming	17:10	-2.73	19:12	-0.7
Outgoing	19:40	-0.23	23:22	3.47
27 April 2014				
Incoming	18:46	-1.50	21:12	0.93
Outgoing	21:52	1.60	00:56*	4.67
28 April 2014				
Incoming	18:12	-2.67	20:46	-0.10
Outgoing	22:06	1.23	23:38	2.77

Table S2. Thresholds and parameters used to detect single targets (Split Beam Method 2, Echoview V5.4, Echoview Software Pty Ltd, Australia) and fish tracks (Alpha-Beta, Echoview V5.4, Echoview Software Pty Ltd, Australia).

Parameter	Value
Single Target Detection	
TS threshold (dB)	-46.5
Pulse length determination level (dB)	13.0
Minimum normalized pulse length	0.6
Maximum normalized pulse length	5.0
Beam compensation model	Simrad LOBE
Maximum beam compensation (dB)	12.0
Maximum standard deviation of minor and major axis angles (°)	1.5
Fish Track Detection	
Minimum number of single targets in track	2.0
Minimum number of pings in track (pings)	1.0
Maximum gap between single targets (pings)	3.0
Major axis weight	30.0
Minor axis weight	30.0
Range weight	40.0
TS weight	0.0
Ping gap weight	0.0
Alpha	0.7
Beta	0.5
Major and minor axis exclusion distance (m)	4.0
Range exclusion distance (m)	0.4

ACKNOWLEGEMENTS

Chapter 4, in full, is a reprint of the material as it appears in Scientific Reports 2017. Rowell, Timothy J.; Demer, David A.; Aburto-Oropeza, Octavio; Cota-Nieto, Juan José; Hyde, John R.; Erisman, Brad E. The dissertation author was the primary investigator and author of this material.