An Acoustic Survey of Grouper Sound Production at Riley's Hump, Tortugas South Ecological Reserve

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Abstract

Long-term passive acoustic recorders were used to monitor spawning habitat use by grouper on Riley's Hump, a fish aggregation site located within the Tortugas South Ecological Reserve. Recordings were made at 10 minute intervals at up to seven sites during portions of 2010 – 2012. Six recording sites were located on Riley's Hump proper in approximately 30 meter depths and a single site was located in deeper adjacent water of 58 meters. Audio/video recorders were deployed overnight at some sites during research cruises in the winter/spring to help identify sources of sound. Sound production of red grouper and red hind were recorded year round and at all times of the circadian day, but were most abundant near nightfall during the winter/spring spawning period. These patterns are consistent with results of previous studies which documented the use of sound production association with reproductive behavior at spawning sites. Evidence of sound production by black grouper was also recorded and identified with video. Black grouper produced at least two variations of a low frequency, modulated tonal call which ranged between 60-120 Hz, lasted on average 5.2 seconds and reached 154-159 dB SPL (re:1 μ Pa). Grouper sound production was concentrated in the frequency range of <200 Hz. Distinct diel and seasonal patterns in sound production were also evident in the 300-400 and 600-800 Hz range but sources of sound production in these frequency ranges were not identified. The results of this study demonstrate that red grouper, black grouper, and red hind use Riley's Hump as spawning habitat.

Introduction

Many groupers are especially important commercial and recreational species and as such there is an ever-increasing focus on the conservation and management of their stocks and habitats. Fundamental to making informed management decisions on these issues is requisite information about population abundance and distribution, life history, and habitat. Data on these topics are typically generated through long-term time series based on visual surveys, catch-and-release studies or subsampling of commercial catches with the main research goals of estimating abundance, change in population size, and size at age - especially at maturity. Recognition and protection of critical habitat also plays a major role in the sustainability of grouper and other reef fish species and this has been realized through the establishment of marine protected areas and reserves. One important consideration in the selection of a location to dedicate as a marine reserve is whether it is used as a spawning aggregation site. In many cases aggregation sites were first discovered by commercial fisherman and later established as marine reserves when their conservation value was realized. Such sites become good locations for establishing long-term field studies to evaluate the efficacy of the reserve for population recovery and to learn more detail about the behavior and general dynamics of spawning aggregations (Burton et al., 2005).

Groupers, as the name implies, form seasonal spawning aggregations at traditional sites. The structure and size of these aggregations varies by species and may directly influence their vulnerability to overfishing. For example, Nassau grouper and red hind form fewer but larger aggregations (Whaylen et al,. 2004; Kadison, et al., 2009) which increase the risk of recruitment overfishing while red grouper appear to have more discrete spawning groups located at individual male territories sometimes defined by pits excavated in the sediment (Coleman et al., 2010; Nelson et al., 2011). A variety of species may overlap at the same location resulting in seasonal reproductive communities and enhanced site value for conservation and research purposes. Of the various conservation strategies applied to grouper populations, the establishment of marine reserves offers the most comprehensive and likely effective approach to management of these fishes (Huntsman et al., 1999; Ault et al., 2008).

In addition to the traditional methods used to study fish populations at spawning aggregation sites passive acoustics represent a relatively new approach. Sound production is common among many fishes and is most often associated with courtship and spawning behavior (Mok and Gilmore, 1983). Acoustic time series of fish sound production can therefore be used as a proxy to document the timing and location of reproductive behaviors. Available recording technologies now allow multi-year deployments during which data may be recorded every few minutes. Deployment durations can vary considerably because recorders are fully programmable.

To date, most passive acoustic research has been conducted on sciaenids and to a lesser extent on serranids (Locascio and Mann, 2011; Mann et. al, 2010). Research has also been conducted on sound production and auditory sensitivity of pomacentrid and batrachoid fishes (Mann and Lobel, 1997; Fine and Lenhardt, 1983). Because sounds are species-specific once the source has become positively identified the information can be referenced to all future recordings (geo-graphic variations of a species sound may exist but are not yet demonstrated). A great deal of field recordings have been made of sounds whose sources remain unidentified. This represents a challenge because of the great many fishes that likely produce sound, most do so mainly at night.

Tags capable of recording sound will be effective at identifying many sources which currently remain unknown.

The most commercially important fishes currently being studied with passive acoustic methods are the groupers, yet research accomplishments are still few. Only the sounds of goliath grouper (*Epinephelus itajara*), red hind (*Epinephelus guttatus*), red grouper (*Epinephelus morio*), and yellowfin grouper (*Mycteroperca venenosa*) have been positively identified and published (Mann et al., 2008; Mann et al., 2010; Nelson et al., 2011; Scherer et al., 2012) and only one study has attempted to quantify population size of red hind using passive acoustics (Rowell et al., 2012). Anecdotal information from divers exists for the Nassau grouper (*Epinephelus striatus*) sound production. Many other grouper species are likely to produce sound and these await discovery.

The purpose of this study was to document grouper use of spawning habitat, using sound production as a proxy for reproductive behavior, at Riley's Hump – a fish aggregation site in the Tortugas South Ecological Reserve (TSER). Riley's Hump was a productive commercial fishing ground particularly for mutton snapper prior to the establishment of the TSER in 2001. Since the inception of the TSER, Riley's Hump supports the recovery of mutton snapper which use it as a spawning aggregation site during the late spring and early summer months (Domeier, 2004; Burton et al., 2005). Many species of grouper also inhabit Riley's Hump but their use of it for reproductive purposes has not been documented.

Methods

Study Site

Riley's Hump is a geologic feature approximately 10 km^2 in area which marks the western extent of the south Florida reef tract and lies entirely within the TSER. The limestone composition is typical of the sedimentary geology of the Gulf of Mexico and its surface ranges from sandy bare areas to rugose hard bottom and low-relief outcroppings. Depths range from approximately 30 meters on the hump proper to approximately 60 meters immediately adjacent to it. Relief is generally higher along the edges, especially from the northeast to southern edges in a clockwise direction. The steepest vertical drop-off is located along the south-south-western edge which is also regarded as having the relatively highest fish densities (Burton et al., 2005). The benthic community is represented by hard and soft corals, gorgonians, and a variety of sponges (Weaver et al., 2006).

Programming, Deployment and Recovery of Acoustic Recorders

Passive acoustic Digital Spectrum recorders (DSG) were deployed at 7 locations on Riley's Hump during portions of 2010 through 2012 (Table 1). Sites included 3 traditional sampling locations where visual surveys of fishes are conducted along with 4 new sites established for this study including a site off the western side of RH in approximately 58 m depth (Figure 1). DSGs were programmed to record 10 seconds of sound every 10 minutes at a 15,094 Hz sample rate and were moored to steel rebar (8'long x 1" diameter) that had been pounded approximately 3' into the limestone. All recorders were deployed and recovered using SCUBA, except for the deployment of the deep water recorder which was dropped in a weighted housing from the swim platform of the M/V Spree, from which all field operations were conducted.

Analysis of Acoustic Data
Each 10 second acoustic file was analyzed in MATLAB v. R2009B with a fast Fourier transform (FFT) to generate a power spectrum from which the band sound pressure level in 100 Hz wide bins was calculated. In some cases, data were analyzed in narrower or wider bin sizes to view patterns in more precise ranges used by some species. The power spectrum plots were examined for patterns in daily and seasonal periods indicative of fish sound production in frequencies below 1000 Hz. Acoustic time series data were examined for peaks associated with lunar phases during the documented winter/spring spawning season of some grouper species in the Gulf of Mexico and Caribbean. Adobe Audition was used to manually review spectrograms of acoustic recordings to identify which species were present and previously undescribed calls.

Deployment and Analysis of Audio/Video Data from Remote Camera Systems
Custom underwater audio/video systems were used to try to verify sound production by grouper species (and other fishes) not yet documented and to gain insight into the behavioral context of sound production. The recording system included a low-light black and white flat board security camera and two hydrophones all of which recorded to a Chase-Cam deck. The system was enclosed in a waterproof housing with batteries and set overnight at select sites where long term acoustic recorders were deployed. The A/V system was capable of recording continuously to compact flash memory for approximately twenty hours. Audio and video data were reviewed with Corel Video Studio and Adobe Audition software.

Results
Patterns in fish sound production recorded at all sites could generally be classified into three main frequency ranges; <200 Hz, 300-400 Hz, and 600-800 Hz.

Frequency category < 200 Hz:
Sounds produced in this frequency range were associated mainly with grouper and exhibited a general diel pattern of increased levels during late afternoon and early evening and lower levels during the day time. However, sounds produced by groupers were recorded at all times of the circadian day. While these results demonstrated patterns in fish sound production in a frequency range used by grouper, manual review of the data was necessary to identify which species were present and whether previously undocumented sounds/species contributed to sound pressure levels recorded in this frequency range.

Spectrographic review of acoustic data confirmed red grouper (*Epinephelus morio*) and red hind (*Epinephelus guttatus*) sound production at all sites. Positive identification of these species was based on previously published descriptions of their sounds (Mann et al., 2010; Nelson et al., 2011). A previously unidentified call type was also commonly recorded at all sites. Two variations of this call were recorded, BGV1 and BGV2. BGV1 was composed of a relatively long frequency modulated tonal portion only and BGV2 was composed of an initial, shorter duration frequency modulated tone followed by several individual pulses which then concluded with the longer frequency modulated tonal portion of BGV1 (Figures 2A – D). The longer frequency modulated tonal portion common to both variations had a mean duration of 5.2 seconds (S.D. = 1.2, n=20), and was modulated between 60-120 Hz at a mean rate of 0.17 seconds (S.D. =0.03, n=20). The highest root mean square (RMS) received sound pressure level (RL) for the longer duration frequency modulated portion of the call was estimated at 159.3 dB SPL. The highest RMS RL for the introductory portion of the call only and the overall combined portions of the call were 153.3 and 154.2 dB SPL (re:1µ Pa), respectively.

This call type was identified 76 times in the audio track of the recordings made with the remote camera systems. In a review of the corresponding video segments black grouper were seen in the video18 out of 76 times the call was made and on 10 other occasions appeared within 25 seconds of the call being made. Of the remaining 48 times the call was made, no grouper were seen in the video. Black grouper was the only grouper species seen on video at or near the time this call was produced.

In most cases only one black grouper appeared in the video when this call was made and on only one occasion was courtship behavior observed between two fish (site 12A). On this occasion, one fish with a blotched pattern, deliberately approached another more monochromic fish from below and behind and appeared to briefly make contact as it passed under the rear portion of it. The two fish then swam slowly in opposite directions out of the video frame (Figures 3A-D). The larger blotch patterned fish swam towards the camera and out of the frame and within ten seconds a relatively high amplitude BGV1 type call was recorded. This form of courtship behavior by black grouper was described by Paz and Sedberry (2008) who studied their spawning aggregations in Belize. These authors also noted that the blotched color phase was seen during the morning of the day of a spawning event.

Other low frequency pulsed and modulated tonal sounds were occasionally encountered during manual review of acoustic files but none were repeated or as commonly encountered as those of the species described. One recording appeared to resemble the pulse train call type of the yellowfin grouper (*Mycteroperca venenosa*) (Scherer et al., 2012) however only one example of this call type was found and the other tonal, frequency modulated call types reported for this species were not discovered in the acoustic records.

Sound pressure levels and periodic diel patterns in the <200 Hz frequency range were strongest during January through early May at all recording sites during 2011 and 2012. However, sound production by each grouper species was recorded year round, though much less commonly so outside of the winter/spring season. Diel variability in sound pressure levels during the winter/spring period ranged from about 5 to 15 dB SPL (re: 1 µPa) above daily background levels for all sites and both years. Diel increases in sound pressure levels on RH did not typically exceed 10 dB above background levels during peak times of grouper sound production and this varied little between sites on the hump. Sound pressure levels recorded at the deep water site typically reached higher amplitudes of +15 dB SPL (re: 1µPa) above daytime background levels and also demonstrated a positive association with the last quarter moon phase. Overall sound pressure levels in this frequency range were contributed to by all three grouper species at this site, but more so by red hind (Figures 4A and B).

Frequency Category 300-400 Hz

Sounds production in the 300-400 Hz frequency range were dominated by a pulsatile sound type characterized by a crepuscular pattern with higher levels (approximately 6-8 dB above background) reached during the evening than in the morning. The seasonal period of sounds produced within this frequency range began in the early spring and continued through late summer /early fall and did not correspond to a lunar period (Figures 5A and B). The timing and levels of sound within the 300-400 Hz range was similar among sites and between years. The source of these sounds was not identified.

Frequency Category 600-800 Hz

Sound production within the 600-800 Hz category was also dominated by a pulsatile call type. Some energy associated with this call extended above and below the 600-800 Hz range but was minimal by comparison. This call was considered to be from a different source than the 300-400 Hz signal because plots of each demonstrated they were out of phase with each other (e.g. not temporally synchronized). This call had a lunar period associated with it which began on or within 2 days of the full moon and continued to about the first quarter moon of the following lunar cycle, a period of approximately 18-20 days. Maximum sound pressure levels, during peak season of this call, were about 25 dB above daytime background levels and were reached half way through each cycle. During each cycle, sound pressure levels increased and decreased normally (Figures 6A and B). This call type occurred at all sites between late March and mid-July. Although this signal was quite prominent it occurred mainly after nightfall and its source was not identified. It may possibly have been generated by a member of the family Holocentridae.

Discussion

In this study we documented sound production by three grouper species at Riley's Hump including red grouper, black grouper, and red hind. Patterns in sound production by red grouper and red hind were similar to those reported previously from recordings made during the winter/spring spawning season at aggregation sites in the Gulf of Mexico and Puerto Rico (Nelson et al., 2011; Mann et al., 2010). In each of these prior studies observations of behavior associated with sound production did not include actual spawning, but rather courtship interactions and territorial patrols during which the presumed male was the sound producer. We did not observe courtship, spawning or territorial behavior by these species during dives nor was it recorded by the remote camera systems. However, the precedent set by the two previous studies qualifies the interpretation of our recordings as documentation of spawning habitat use by these species at Riley's Hump.

Sound production by black grouper has not been previously described. The calls we attribute to black grouper recorded in this study are consistent with the stereotypical characteristics of grouper calls (low-frequency, modulated, and long duration) but are unique in that they do not contain the frequency down-sweep common to calls of the red and yellowfin groupers and red hind. Although we observed courtship behavior followed closely by this call type in only one video segment, the high coincidence of black grouper as the only grouper species appearing in the video at or near the time this call type was produced makes us confident that it is the source. Additional data collected from documented black grouper spawning locations to corroborate this conclusion would however be useful. Black grouper spawning aggregation sites are not well documented in the U.S. In fact, we are aware of only one publication that reported the formation of a probable spawning aggregation at a site in the Florida Keys Marine Sanctuary (Eklund et al., 2000). In addition to demonstrating that Riley's Hump is likely a spawning site for black grouper, the information learned from this study about its sound production can be used to help document and protect other spawning aggregation sites while also providing opportunity to learn more about the role of sound production by this species.

The combination of these three grouper species using the same spawning aggregation site is interesting because it represents a broad range of aggregating behaviors. For example, on one end of this spectrum, red grouper are not believed to migrate long distances or form large spawning aggregations but instead form small polygynous groups over wide areas (Coleman, 2010). Evidence from Belize indicates that black grouper form numerous medium sized aggregations (<200 individuals) at various locations among offshore atolls (Paz and Sedberry, 2008). By comparison, red hind traditionally form fewer, large (possibly >1000) transient spawning aggregations of individuals that migrate long distances at specific times (Nemeth, 2012). In general, species which perform aggregation behavior like that of red hind are most at risk of overfishing. For this reason knowledge of red hind spawning locations have been an important consideration in the establishment of marine protected areas and seasonal closures in the U.S. Caribbean and so it is especially fortunate to have discovered this previously undocumented aggregation at Riley's Hump. Sonic tagging of groupers at Riley's Hump during the spawning season could provide useful information on whether they are resident or transient and what their geographic range is, especially in regard to the reserve boundaries and what level of connectivity may exist among regional populations. Farmer and Ault (2011) found home ranges of black and red groupers within the Tortugas North Ecological Reserve and Dry Tortugas National Park limited to 7.6 km^2, an area smaller than Riley's Hump.

While most sound production by each grouper species reported here occurred during the winter and spring, calls were also recorded at other times of the year. A manual review of spectrograms from months outside the spawning season indicated this occurred more often by red grouper. It is difficult to conclude an alternative meaning from this without information from observations of behavior. In general though, this may mean that spawning occurs during other times of the year. Black grouper, for example have been reported to remain in sexually mature condition year round (Crabtree and Bullock, 1998). It is also possible, and more likely, that the context of sound production is associated with other forms of behavior as well. Red grouper for example, excavate and maintain pits in the sediment and are believed to have strong site fidelity. This scenario may warrant the use of sound production in a territorial or agonistic context.

The source of sounds produced in the 300-400 and 600-800 Hz ranges remain unidentified but may be associated with members of the family pomacentridae and holocentridae, several species of which are common at Riley's Hump. The seasonal and lunar timing of the 600-800 Hz sounds do overlap somewhat with the May-July mutton snapper reproductive aggregation period at Riley's Hump. However, on many occasions where large schools of mutton snapper were recorded by remote camera systems, including courtship and spawning behavior, this sound type was not coincidentally recorded. The fact that these signals appeared so dominantly in the acoustic record yet their source remains unidentified is an example of the challenges which remain in passive acoustics.

Riley's Hump is a documented spawning aggregation site for mutton snapper and courtship behavior of other species has also been observed by divers. The results of this study provide additional documentation of the importance of Riley's Hump as a spawning ground and therefore source of larval recruits for populations of these three commercial grouper species. Domeier (2004) demonstrated the potential of Riley's Hump as a source location of mutton snapper larvae which recruit to juvenile habitat throughout the Florida Keys and southeast Florida. This larval pathway would presumably be used by any species originating at Riley's Hump and recruiting to suitable juvenile habitat in the region. Ault et al. (2008) reported spawning potential ratios (SPR) of 0.8% and 17.7% for black and red grouper in the Florida Keys, respectively. These values are far below the federally defined benchmark of 30% for sustainability of these species, especially for the black. Data for red hind were not found but are also expected to be below this standard. The use of Riley's Hump as reproductive habitat for these overfished species further underscores its importance as a marine reserve.

Remote monitoring with passive acoustics was especially useful for documenting spawning habitat use in the adjacent deep water which relatively little is known about. Long-term, high resolution monitoring of reproductive behavior by sound producing fishes at aggregation sites can be achieved with these methods at very low cost in comparison to traditional methods. Future research should focus on sound-truthing the source and behavioral context of sound production by other species to increase the value of passive acoustics as tool for monitoring reproductive habitat use and efficacy of marine reserves.

Acknowledgements
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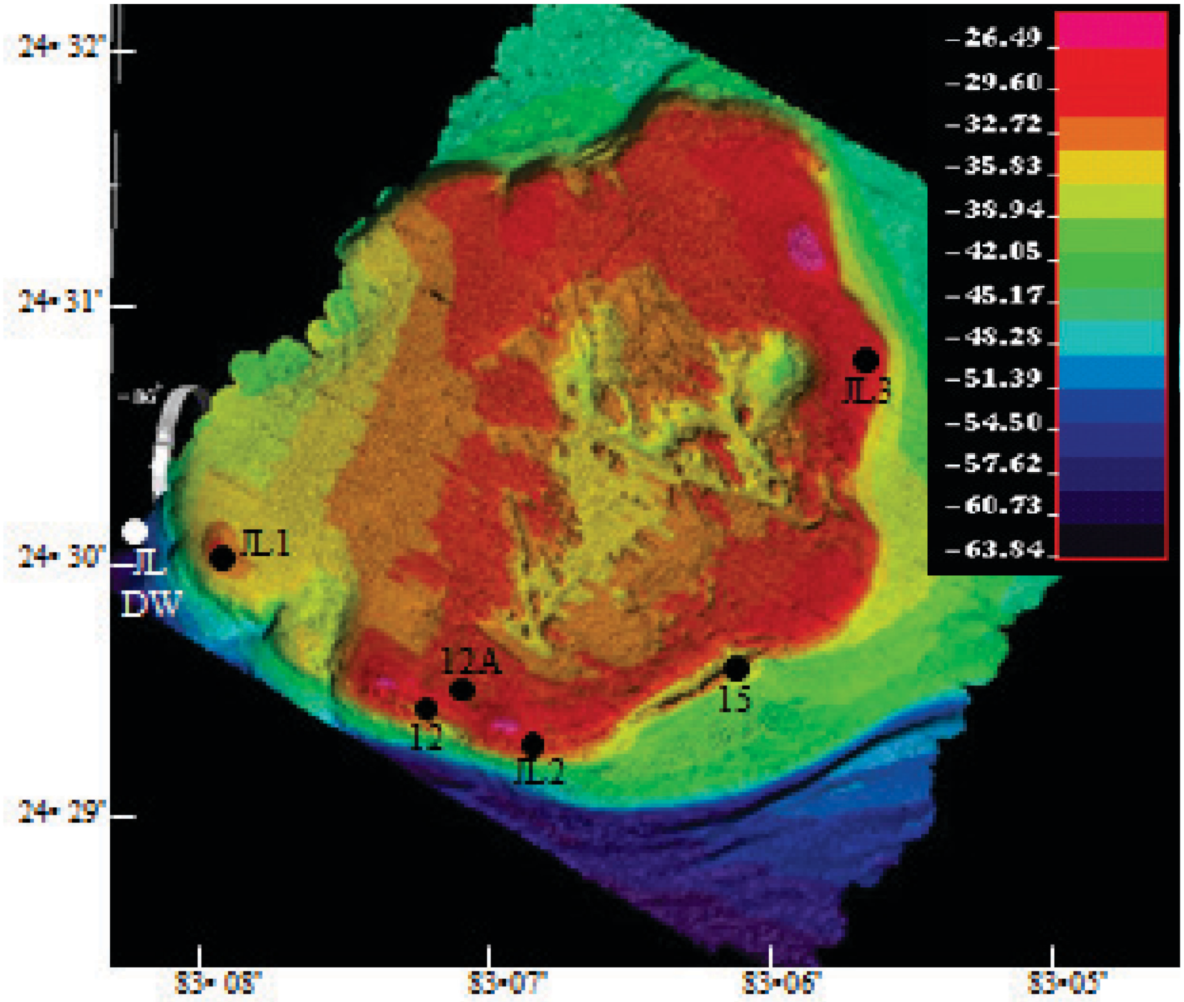
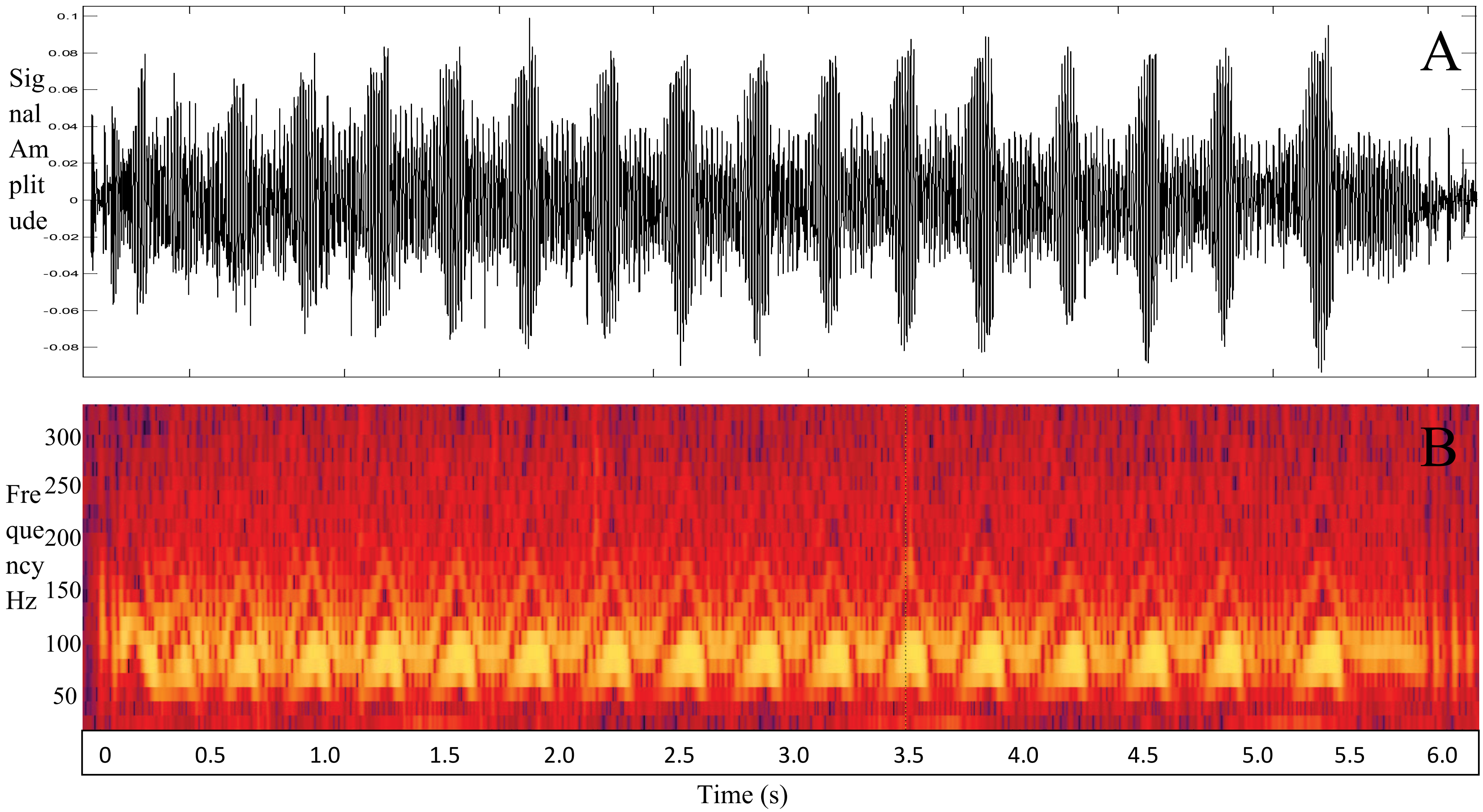


Figure 1. Study site locations on Riley's Hump where long-term acoustic recorders were deployed for periods during 2010-2012. The JLDW site in white font on the left side of the image is located farther west than indicated on the figure, and lies in approximately 58 meters depth. Water depths at all sites located on the Riley's Hump proper are approximately 30 meters.
**reprinted from Burton et al., 2005*



Figures 2A and 2B: (A) Waveform and (B) spectrogram of black grouper call type variation 1 (BGV1). The call is frequency modulated between 60-120 Hz at a rate of about 0.2 seconds. Average duration of this call type was 5.2 seconds (n=20) and highest received levels recorded were 159 dB SPL (re: 1µ Pa).

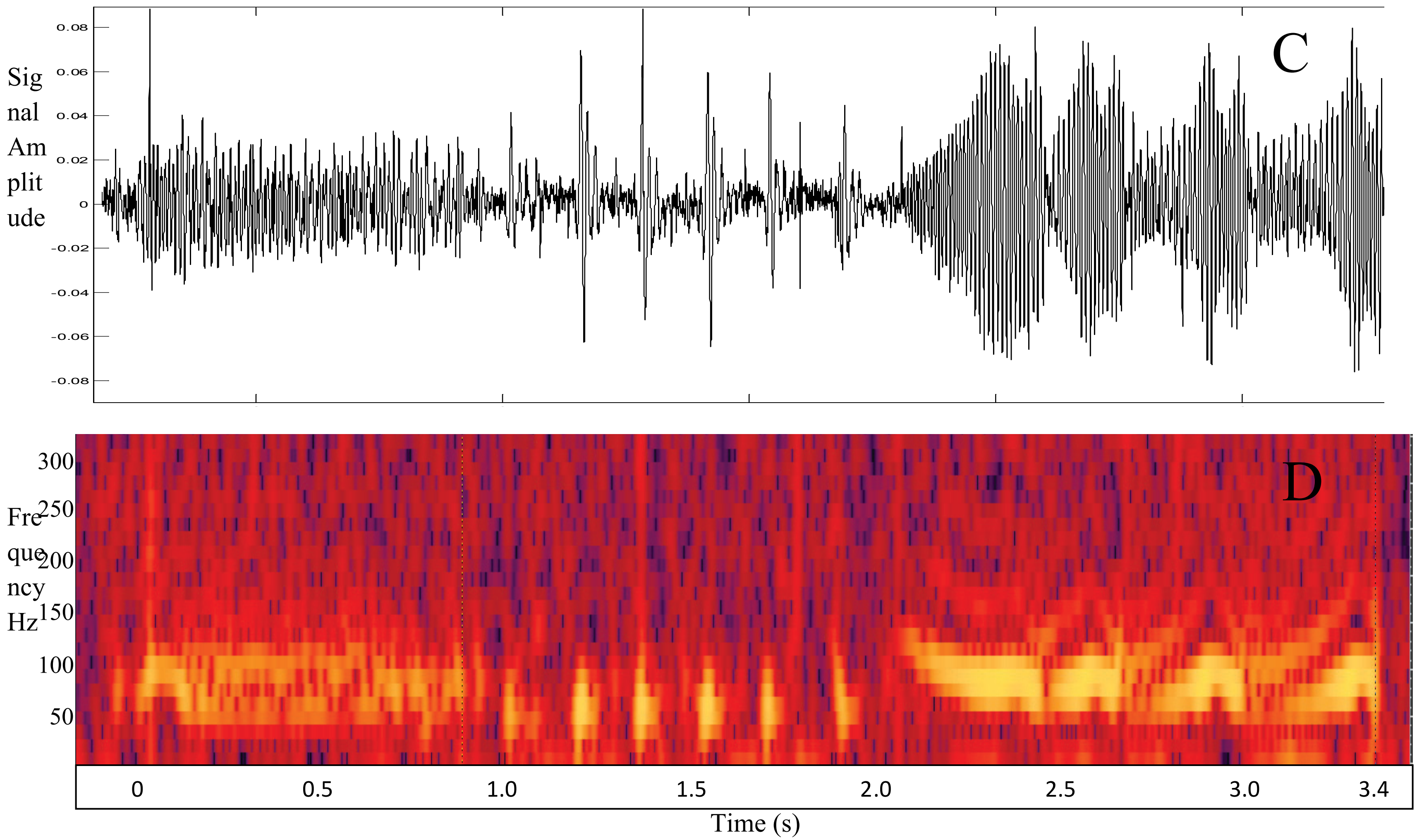


Figure 2C and 2D: (C) Waveform and (D) spectrogram of black grouper call type variation 2 (BGV2). The call contains an introductory portion composed of a short frequency modulated period followed by individual pulses and then the longer frequency modulated portion common to BGV1. The BGV2 variation was uncommon in field recordings. How the context of the two call variants differs is unknown. The longer frequency modulated end portion of this call appears shorter because the programmed 10 second recording period happened to end during the call.

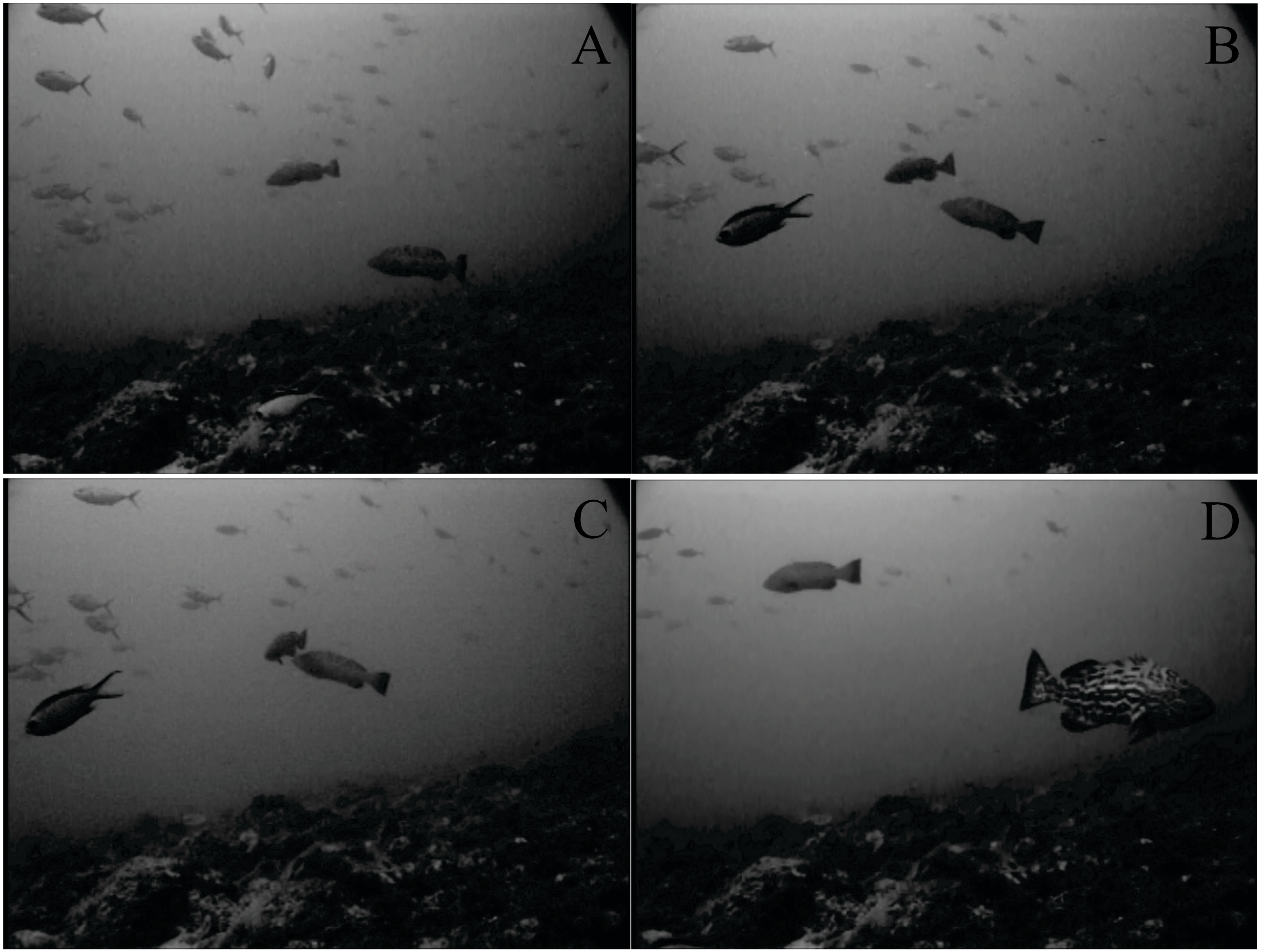


Figure 3A & B. Site 12A, 4/27/2010. A blotch patterned black grouper approaches a second black grouper from below; 3B. The approaching fish passes directly beneath the rear portion of the second fish and briefly makes contact, but no additional interaction occurs; 3C.The fish swim away from each other in opposite directions and a BGV1 call type is produced seconds later. When this camera system was deployed divers reported seeing 'several' black grouper up in the water column swimming together in a generally circular pattern.

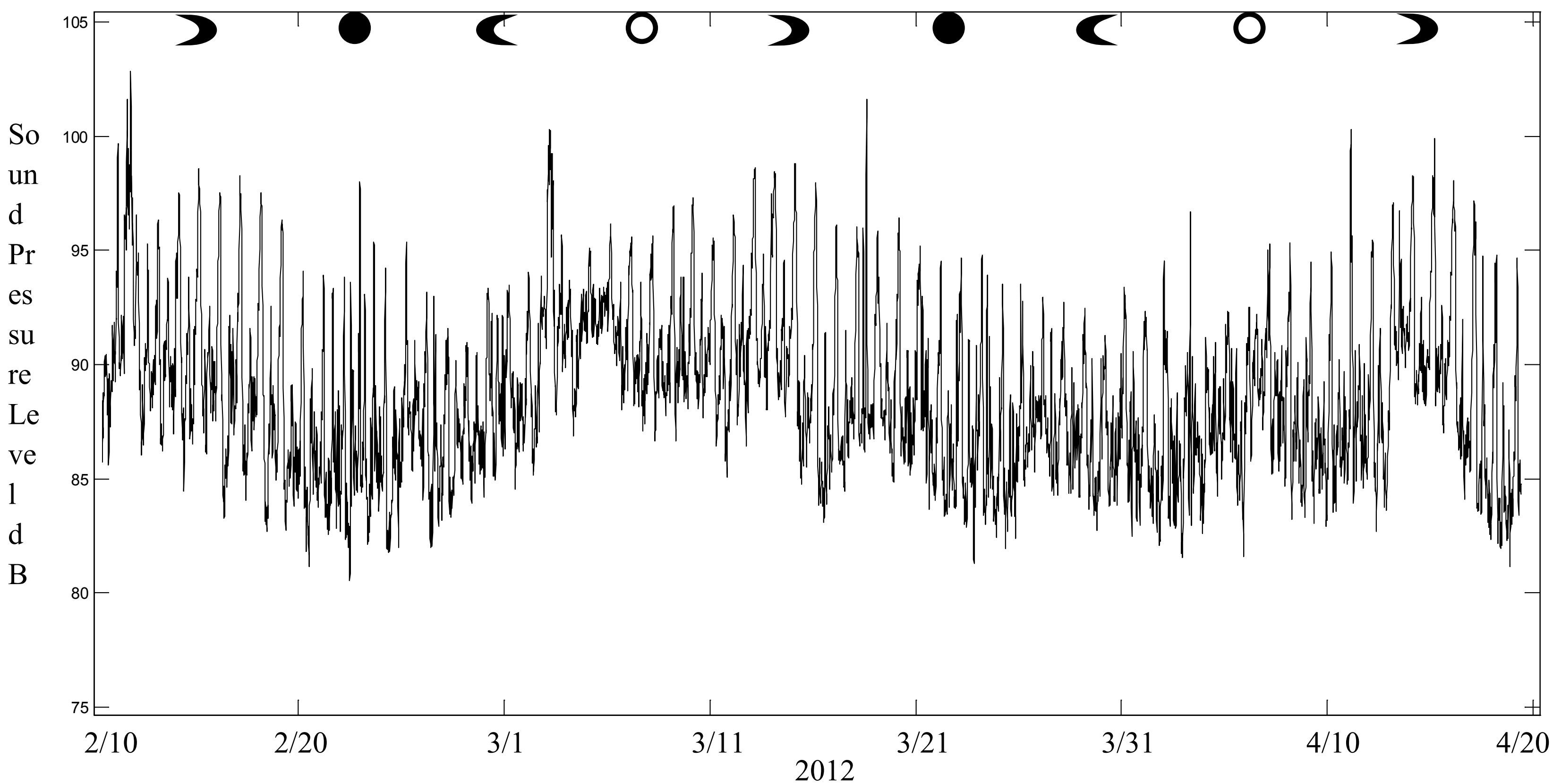


Figure 4A: An example of seasonal periodicity in the acoustic time series data for the <200 Hz frequency category recorded at the deep water site, DWJL. All three grouper species (red, black and red hind) were recorded at each site, but red hind appeared more dominant at the deep water site shown here. Sound production occurs on all lunar phases but peaks are coincident with the last quarter moon phase. Sound production occurs throughout the day but diel patterning is evident as nightly peaks interspersed by day time troughs; a pattern illustrated more closely in figure 4B. = last quarter moon, = new moon, = first quarter moon, = full moon.

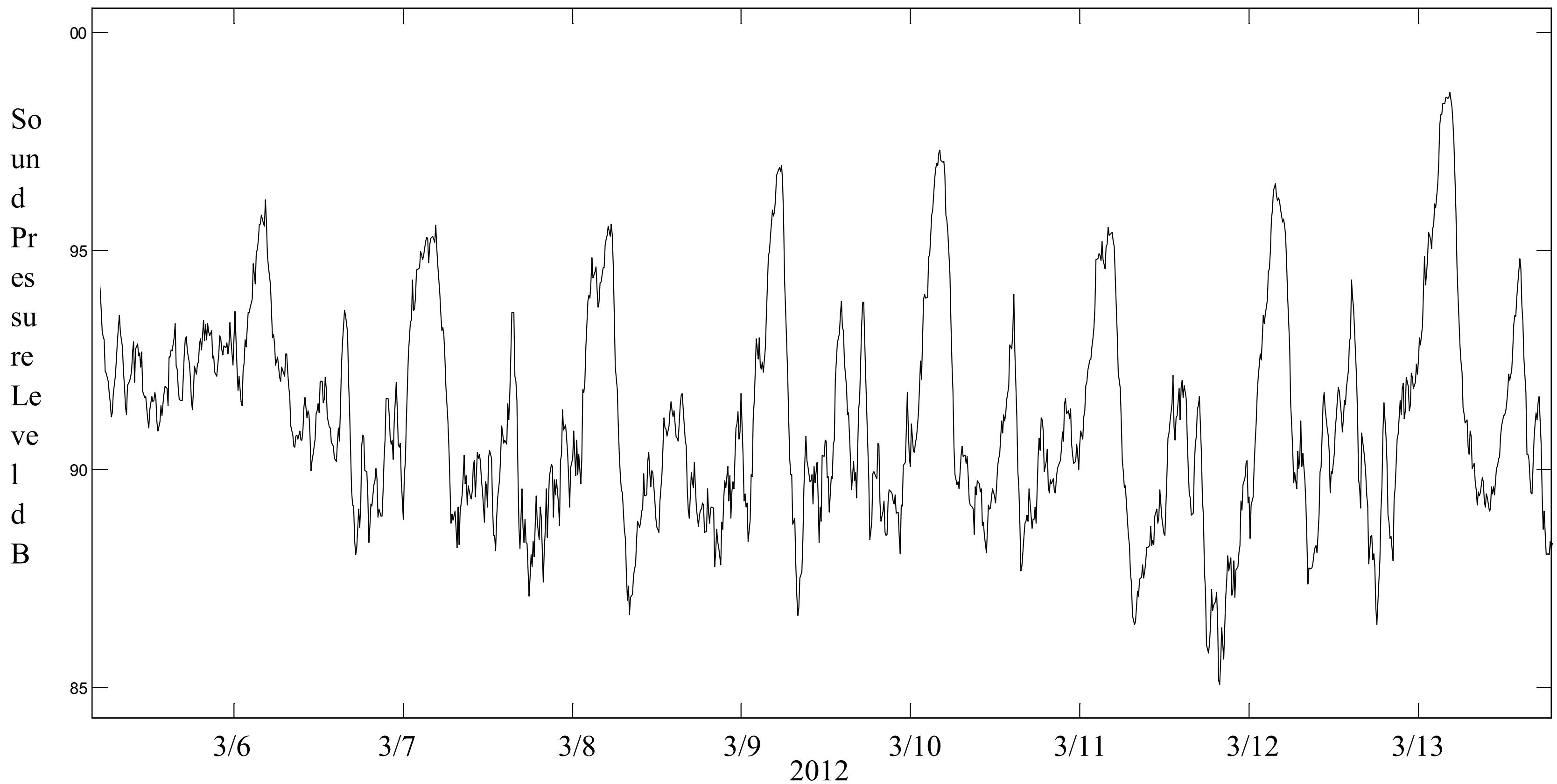


Figure 4B: An example of the diel periodicity of signals recorded in the <200 Hz frequency category at the deep water site DWJL. Calls were recorded by each of the three grouper species at all times of day but were distinctly greater in the late afternoon/early evening as nightfall approached.

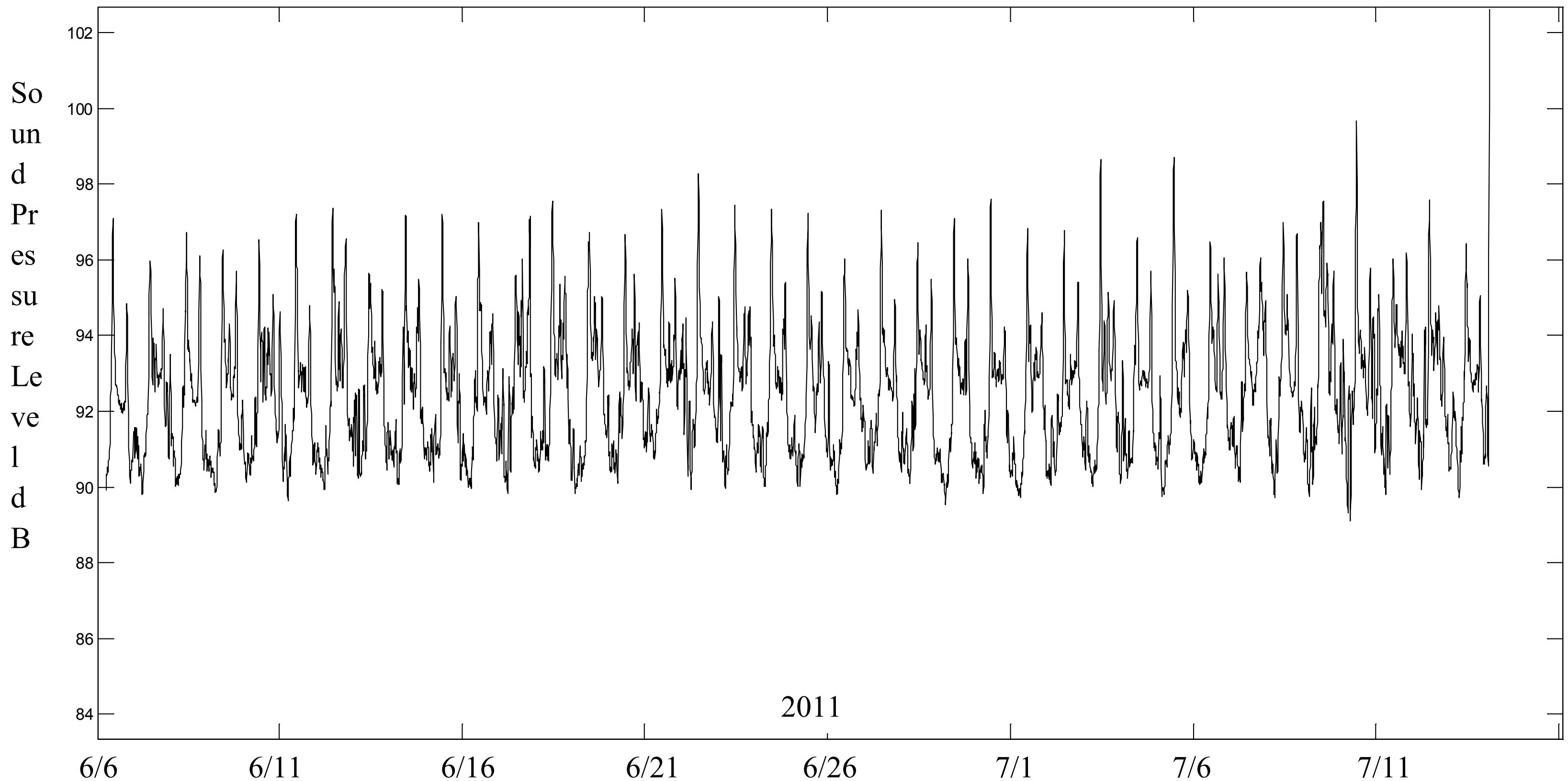


Figure 5A: An example of seasonal periodicity in the acoustic time series data for the 300-400 Hz frequency category recorded at site JL1. The seasonal period of sound production in this frequency range occurred from early spring through late summer/early fall, well beyond the limits of the x-axis. Diel periodicity is defined by a crepuscular pattern illustrated more closely in figure 5B. No lunar periodicity was evident in this frequency range. The fish species responsible for sound production in this time series ~~are~~ remain unidentified.

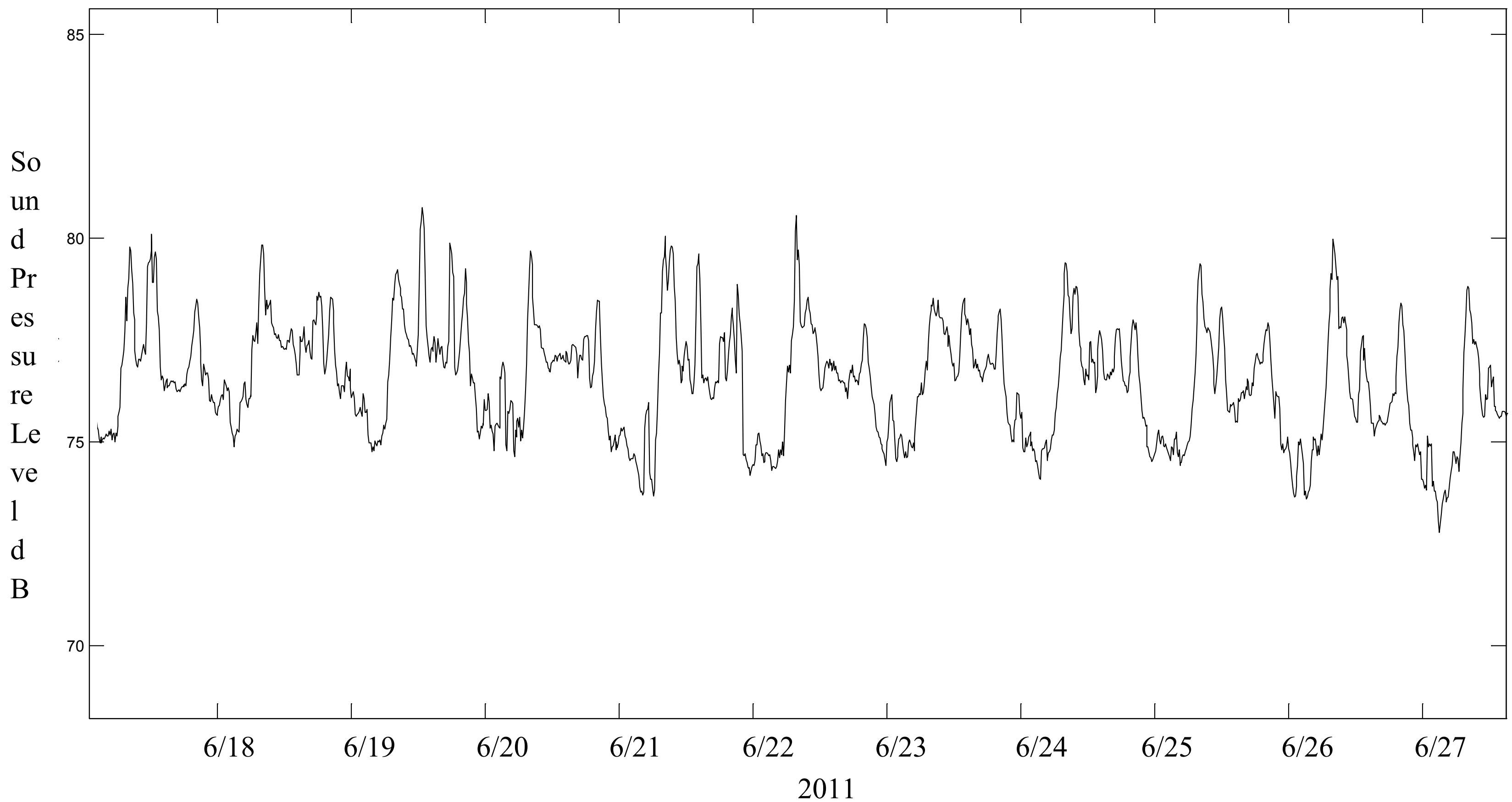


Figure 5B: An example of the diel periodicity of signals recorded in the 300-400 Hz category recorded at site JL1. Sound production in this frequency range was distinguished by a crepuscular pattern with higher levels occurring near nightfall and slightly lower levels in the morning. This is the only frequency category that demonstrated a crepuscular pattern in sound production.

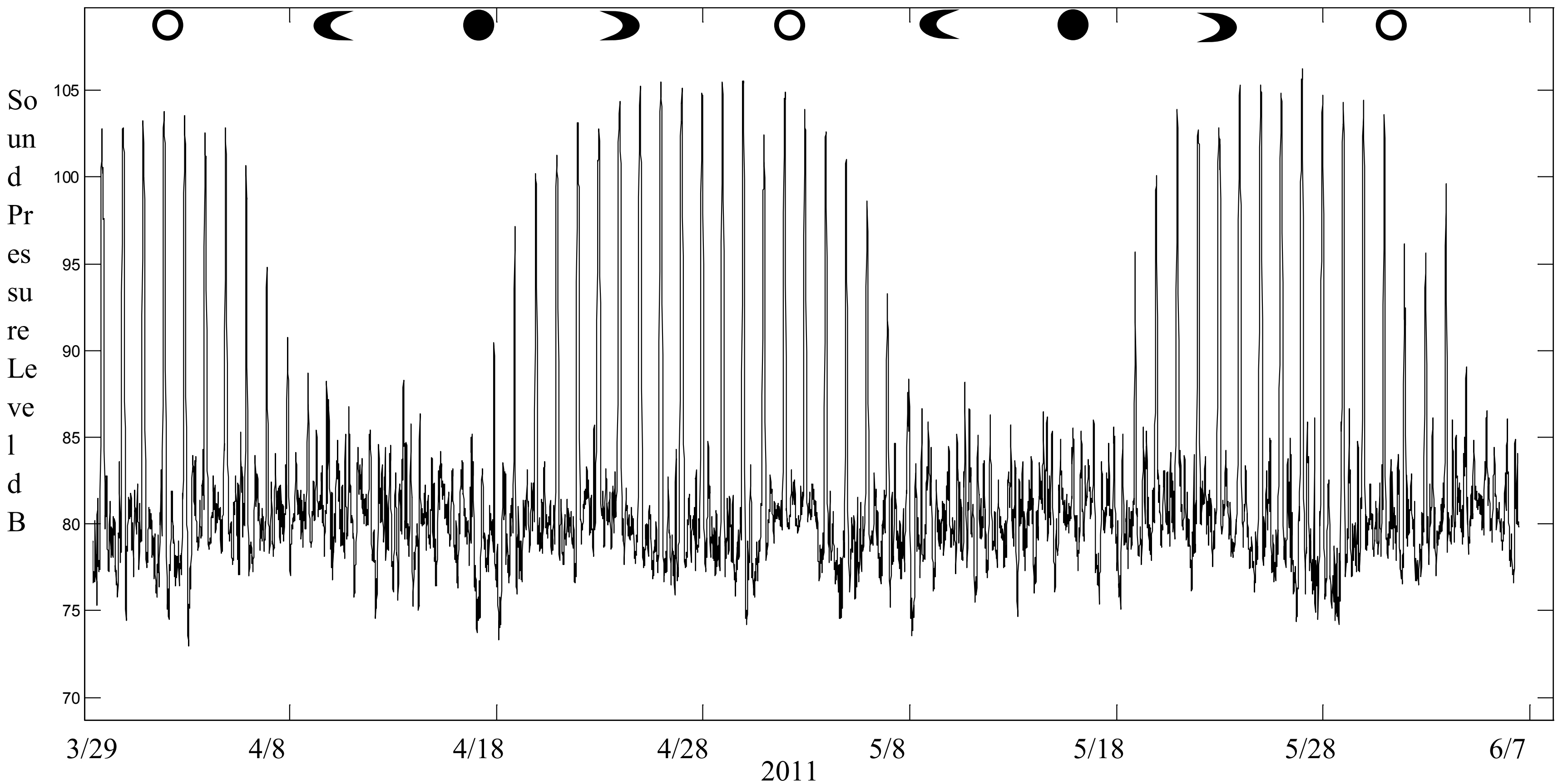


Figure 6A: An example of seasonal periodicity in the acoustic time series data for the 600-800 Hz frequency category recorded at site JL2. The seasonal period of sound production in this frequency range occurred from early spring through mid-summer. Sound pressure levels increased and decreased gradually over a period of about 20 days beginning near the new moon. The species responsible for sound production in frequency range was not identified however; members of Holocentridae (squirrelfishes) may be associated with this signal.

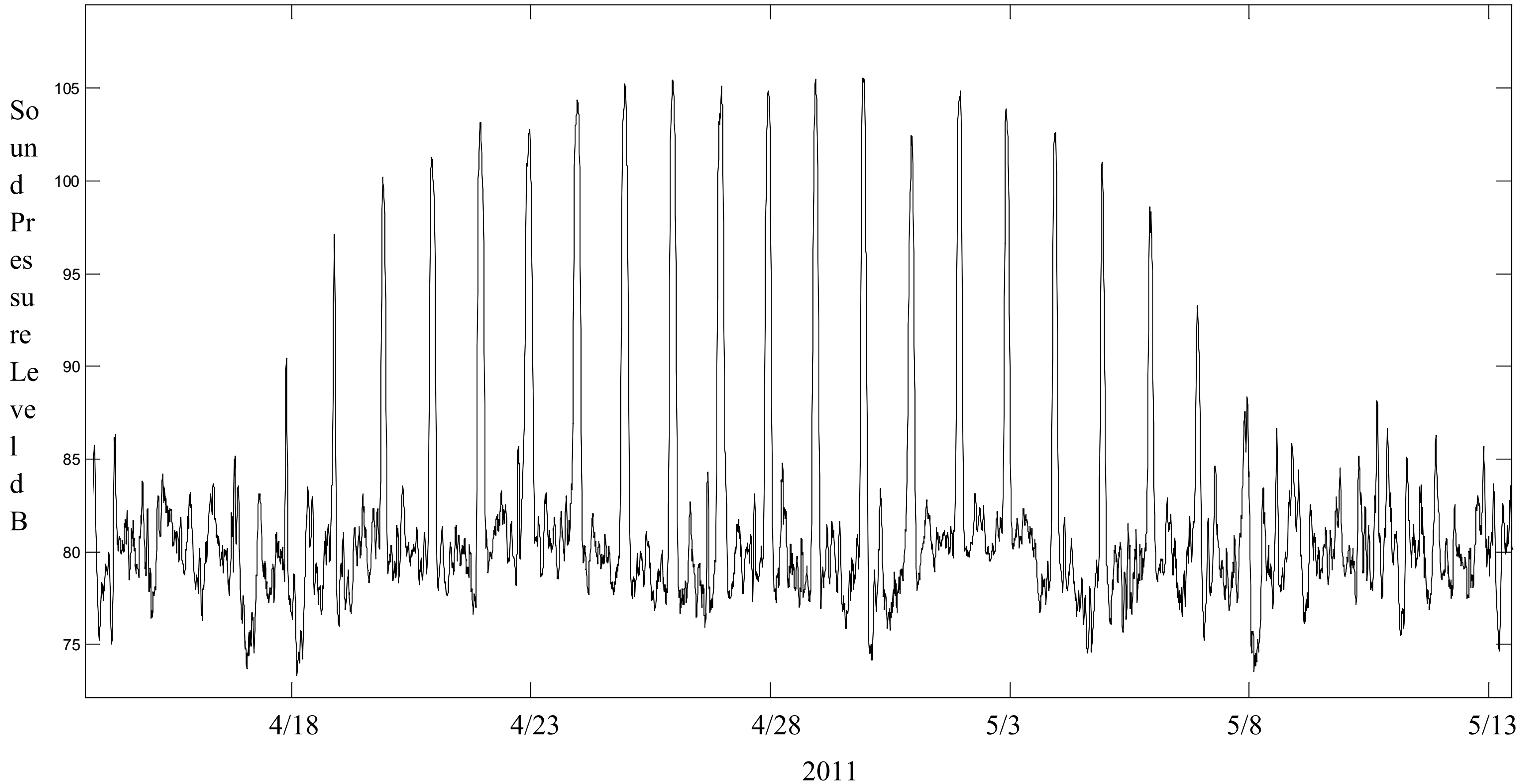


Figure 6B: An example of the diel periodicity of sound production in the 600-800 Hz category recorded at site JL2. Sound production in this frequency range began near nightfall and lasted approximately 5-6 hours each night.

Table 1. Deployment record of passive acoustic recorders at Riley's Hump. All recorders were programmed to record 10 seconds of sound every10 minutes at a sample rate of 15,094 Hz.

Site	Deployment Period
12	4/29/2010 - 6/28/2010
12	1/18/2011 - 7/14/2011
12A	4/29/2010 - 7/16/2011
15	4/29/2010 - 12/9/2011
JL1	1/18/2011 - 6/17/2012
JL2	1/18/2011 - 6/17/2012
JL3	1/18/2011 - 6/21/2012
JLDW	7/17/2011 - 6/13/2012