The Role of Backreef Soundscapes and their Spatial Structure for Recruitment of Tropical Marine Larvae

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THE ROLE OF BACKREEF SOUNDSCAPES AND THEIR SPATIAL
STRUCTURE FOR RECRUITMENT OF TROPICAL MARINE LARVAE

by

Emily R. Anderson
H.B.S. June 2014, Oregon State University

A Thesis Submitted to the Faculty of
Old Dominion University in Partial Fulfillment of the
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Approved by:

Mark J. Butler (Advisor)
Jack Butler (Member)
Holly Gaff (Member)
ABSTRACT

THE ROLE OF BACKREEF SOUNDSCAPES AND THEIR SPATIAL STRUCTURE FOR RECRUITMENT OF TROPICAL MARINE LARVAE

Emily R. Anderson
Old Dominion University, 2021
Advisor: Dr. Mark J. Butler

Underwater sound is a cue used by many marine larvae to orient to coastal habitats including backreef, sponge-dominated hardbottom habitat in the Florida Keys (Florida, USA) – a particularly “noisy” coastal habitat. However, the distance over which acoustic cues are attractive to settlement-stage larvae - is generally unknown. I examined this phenomenon in a region of the Florida Keys where mass sponge die-offs have diminished both underwater soundscapes and larval settlement. The absence of pronounced hardbottom-associated sound over such a large area allowed me to experimentally test in situ the response of fish and invertebrate larvae to broadcasted sounds at different distances from their source. I first measured sound recording from healthy hardbottom habitat at seven distances from an underwater speaker to determine the maximum range of the signal. Based on those results, larval collectors were then deployed at 10, 100, 500, and 1000 m from speakers broadcasting sounds recorded at either degraded or healthy hardbottom sites for five consecutive nights during each of three new and full moon periods in summer/fall 2019. Larval settlement onto those collectors was affected by lunar phase and soundscape type, but the effect of distance on larval settlement varied among species and, in most cases, the effect was small and not likely to be ecologically
significant. The absence of a strong larval settlement response to a sound cue lies in contrast to results from other studies. I hypothesize that the small (<500 m) radius of the broadcasted soundscapes may have limited the magnitude of the larval response to locally available larvae and because the experiment within a large, relatively quiet seascape where local larval abundance may have been low. If so, then planktonic larvae may require a series of acoustic “sign-posts”, perhaps in combination with other cues (e.g., chemical), to successfully orient to distant nursery habitats. Although habitat restoration efforts may be able to restore healthy soundscapes, the typically small size and number of restoration sites may limit the range of the acoustic cue and larval attraction to restored habitats.
This thesis is dedicated to my mother, Jacqueline R. Anderson and to my father, Scott W. Anderson for always encouraging my love of science and nature, and for providing continual love and support.
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<tr>
<td>dB</td>
<td>Decibel</td>
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<tr>
<td>FL</td>
<td>Florida</td>
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<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>$I_s$</td>
<td>Power of signal</td>
</tr>
<tr>
<td>$I_N$</td>
<td>Power of background noise</td>
</tr>
<tr>
<td>Pa</td>
<td>Pascal</td>
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<tr>
<td>SNR</td>
<td>Signal to noise ratio (units of dB)</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
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<tr>
<td>V</td>
<td>Volts</td>
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INTRODUCTION

Sound is an integral part of the natural landscape. Animals produce sounds for defensive, agonistic, and breeding purposes, among others. These sounds are in turn, a source of important biological information for other animals. For example, frogs and toads use calls to advertise for mates (Gerhardt 1994), birds produce courtship songs and calls to defend their territory or raise alarms (Catchpole and Slater 1995, Cresswell 1994), and bats use ultrasonic clicks for echolocation (Simmons et al. 1979).

The combination of sounds produced by physical (e.g., waves, wind), biological (e.g., fish, snapping shrimp), and anthropogenic (e.g., ships, drilling) sources emanating from the landscape is known as the “soundscape” (Pijanowski et al. 2011a, 2011b). Different habitats have unique acoustic signatures (McWilliam and Hawkins 2013, Butler et al. 2016) that can vary with time of day (Cato et al. 1978, Radford et al. 2010), temperature (Kaplan et al. 2017), and lunar cycle (Staaterman et al. 2014). Soundscapes reflect the biological assemblage (Kaplan et al. 2015) and can indicate habitat quality. For example, Piercy et al. (2014) found that sound level decreased on degraded coral reefs and Butler et al. (2016) found that degraded hardbottom habitats were quieter than healthy, sponge-rich hardbottom.

In the marine world, sound travels faster and farther than in air (~1500 m/s vs ~340 m/s; Urick 1983). Sound speed increases with increasing pressure, temperature, and salinity and sound propagation can be altered by some physical factors such as substrate type and sea surface state (Urick 1983). Sound waves are comprised of two components: particle motion and pressure. Particle motion is the back-and-forth motion of the particles of the medium and is dominant within 1-2 wavelengths from a source; this is called the acoustic nearfield. Further
from the source, in the acoustic farfield, particle motion decays rapidly and the variation in pressure caused by the compression and expansion of the medium is the dominant component of sound. Particle motion is inherently directional; the particles move back-and-forth in the same direction that the wave is traveling, whereas pressure is not associated with direction (Montgomery et al. 2006) though directionality can be determined with multi-sensor arrays (Aarabi 2003) or organic arrays like ears (Joris and van der Heijden 2019). Thus, sound can transmit information quickly, directionally, and largely unaffected by many environmental factors (i.e., currents, light levels) that can alter other forms of information transmission such as vision or olfaction (Tyack 1998).

SOUND PRODUCTION AND PERCEPTION IN THE MARINE ENVIRONMENT

Given that sound is an effective mechanism for transferring information in the marine environment many marine animals have evolved mechanisms of producing and detecting sound. Marine mammals, fishes, and invertebrates all contribute to the underwater biophony. Whales have a large repertoire of communicative calls (McCauley et al. 2000) and toothed whales and porpoises use echolocation for detecting prey (Wood and Evans 1980, Miller et al. 2004), but their well-known production of sounds comprise only a small part of underwater soundscapes. Most of the biological components of underwater soundscapes are produced by fishes (McCauley and Cato 2000, Locascio and Mann 2008, Tricas and Boyle 2014) and invertebrates (Johnson et al. 1947, Everest et al. 1948, Lillis et al. 2014). Soniferous fishes (e.g., grouper [Serranidae], grunts [Haemulidae], toadfish [Batrachoididae]), produce a range of vocalizations such as whistles, booms, growls, and drums (Gray and Winn 1961, Lobel 1992, Mann 1998, Nelson et al. 2011). These vocalizations are associated with courtship or agonistic interactions.
(Mann 1998, Ladich 2004, Schärer et al. 2014) and are produced by various mechanisms such as stridulation of the jaw or contraction of muscles attached to the swim-bladder (Kasumyan 2008). The popcorn-like crackle that dominates many marine soundscapes is produced by snapping shrimp (Everest 1948, Johnson et al. 1947, Au and Banks 1998, Lillis et al. 2014, Butler et al. 2016) that create cavitation bubbles with their claws to produce their iconic snap (Versluis 2000). Stomatopods (i.e., mantis shrimps) also create a popping sound with their claws by cavitation (Hazlett and Winn 1962) and can produce a low rumble by vibrating their bodies (Staaterman et al. 2011). Spiny lobsters produce a distinctive rasping sound by rubbing the plectrum, an extension at the base of the long second antennae, against a “file” under each eye (Patek 2001, 2002) as a defensive warning (Meyer-Rochow & Penrose 1976, Bouwma & Herrnkind 2009, Staaterman et al. 2010).

The mechanisms of hearing in adult fishes are fairly well understood and although there is substantial variation among species, they share the same basic structures (Popper and Coombs 1980, Popper & Fay 2011). The basic fish ear includes three semicircular canals with associated sensory epithelia and three otolith organs that primarily detect the particle motion component of a sound field. In a sound field, otoliths essentially act as a differential density accelerometer. The fish, which is approximately the same density as the water, moves at the same amplitude and phase as the water while the otolith, which is denser than the fish and therefore has more inertia, moves with a different amplitude and phase than the rest of the body. Sensory bundles attached to the otolith detect the difference and respond as in other vertebrate ears (Popper and Coombs 1980, Popper and Fay 1999, 2011, Ladich 2004). Many fish species also have specializations that link the swim bladder, a gas inclusion inside the fish that vibrates in response to sound pressure, to the inner ear which allows the fish to detect the pressure component of the sound field (Popper
and Fay 1999, Popper and Fay 2011, Ladich 2004). Fish hearing tends to be most sensitive in lower frequencies peaking under 1-5kHz (Popper and Fay 1993, Ladich and Fay 2013, Nedelec et al. 2016) and these same mechanisms and sensitivity likely extend to pre-settlement larval fish stages (Montgomery et al. 2006).

In contrast to fishes, far less research has been devoted to sensory physiology and hearing mechanisms of invertebrates. In crustaceans, small mechanoreceptor hair cells (20µm-2000µm) cover the cuticle and may respond to certain frequencies based on the cell length. Statocysts in decapod crustaceans may have a similar function to the fish otolith (Popper et al. 2001). The statocyst contains a statolith, a small calcareous particle, surrounded by a gelatinous fluid and in contact with sensory hair cells that detect motion of the statolith (Popper et al. 2001). There is limited information about these structures in the early life-stages of crustaceans and how they may contribute to sound perception (Montgomery et al. 2006). For example, Sekiguchi and Terazawa (1997) did not find sensory hairs in the statocyst of spiny lobster (Jasus edwardsii) postlarvae yet they respond to sound (Jeffs et al. 2005, Stanley et al. 2015, Hinojosa et al. 2016). Few studies have examined the hearing sensitivity of crustaceans. Behavioral studies of some crustacean species suggest sensitivity in the range of 5-400Hz (Goodall et al. 1990, Roberts et al. 2016), whereas electrophysiological analyses revealed that a Panopeus crab and a Palaemon prawn were sensitive to sound in the range of 75-1600Hz (Hughes et al. 2014) and 100-3000Hz (Lovell et al. 2005), respectively. There is little published research on sound perception in non-arthropod invertebrates. Squid have statocysts and appear to be sensitive to sound in the same range as fish (Mooney et al. 2010). Larvae of some sessile invertebrates detect and respond to sound, though it is not understood how they detect sound (e.g., oysters: Eggleston et al. 2013,

SOUND AS A SETTLEMENT CUE

Almost all reef-associated fish and benthic invertebrates have a pelagic larval stage (Bradbury and Snelgrove 2001) and most are active swimmers, meaning they do not rely solely on the currents to disperse (Kingsford et al. 2002, Leis 2006, Fiksen et al 2007). But the pelagic larvae of coastal species must locate suitable settlement habitat from afar (Pineda et al 2007, Cowen et al. 2007). Given the hearing capacity of larvae (Montgomery et al. 2006), and the fact that underwater sound propagates long distances (Urick 1983), sound is one of the most likely long-distance cues used by larvae for orientation to coastal habitats (Kingsford et al. 2002, Rogers and Cox 1988, Stobutzki and Bellwood 1998, Leis et al. 1996, Montgomery et al. 2006). Although the mechanisms that allow larvae to detect underwater sound are not completely understood, a number of experiments have demonstrated that fish and invertebrate larvae can perceive and use auditory information.

Fishes: Stobutzki & Bellwood (1998) were one of the first to provide evidence that larval fish actively swim towards reefs using sound as a potential cue. Multiple experiments since then have demonstrated that sound may be an attractive cue for settlement-stage fishes. Light traps attached to speakers broadcasting coral reef soundscapes collected more reef fish larvae and of greater diversity than silent light traps (Tolimieri et al. 2000, Leis et al. 2003, Simpson et al. 2004, Simpson et al. 2008) or light traps broadcasting degraded reef soundscapes (Gordon et al. 2018). Artificial patch reefs in open sandy habitats exposed to healthy coral-reef soundscapes also attract greater settlement and diversity than patches without broadcasted sound (Simpson et
al. 2005) or patches exposed to degraded habitat soundscapes (Gordon et al. 2018). Additionally, Simpson et al. (2005, 2008) found that many coral reef fish genera prefer the high frequency component of reef soundscapes (570-2000 Hz), generally produced by invertebrates, over the low frequency components (<570 Hz), produced by fish. Binary choice chambers have also been used to demonstrate directional movement for several fish species, indicating that fish larvae perceive sound and use it as a navigational cue (Tolimieri et al. 2004, Leis & Locket 2005, Parmentier et al. 2015). Indeed, numerous fish groups are sensitive to auditory settlement cues (e.g., Acanthuridae, Apogonidae, Balistidae, Blenniidae, Chaetodontidae, Gobiidae, Holocentridae, Lethrinidae, Monacanthidae, Mullidae, Nemipteridae, Pomacentridae, Pseudochromidae, Sphyraenidae, Sygnathidae, Thrichonotidae).

Despite the large number of behavioral studies, fewer investigations have examined the physiological mechanisms associated with larval fish sound detection. Wright et al. (2005, 2008, 2010, 2011) measured auditory brainstem response (ABR) in several species of settlement-stage coral-reef fish larvae, which detected sounds in the range of 100-2000Hz and were sensitive enough to detect reef soundscapes at least hundreds of meters away. In general, behavioral assays have yielded higher sensitivity to sound than ABR. The variability in sensitivities between the different types of studies are due to differences in experimental conditions (Sisneros et al. 2016). ABR studies are conducted in a tank where the sound field does not behave as it does in natural settings and threshold estimates are often subjective to the observer (Sisneros et al. 2016). Behavioral assays and field studies, conducted in a more natural acoustic setting, are more likely to detect natural behavior.
Crustaceans: Several species of decapod crustaceans also respond to soundscapes indicative of their settlement habitats. Light traps with broadcasted reef sounds collected more larval crabs than silent traps (Jepps et al. 2003). Five species of coastal crab postlarvae moved towards broadcasted reef soundscapes in an in-situ binary choice chamber, but failed to make a choice in silent controls (Radford et al. 2007). Similar experiments demonstrated that the spiny lobster *Jasus edwardsii* is attracted to the soundscapes of rocky reef settlement habitats (Hinojosa et al. 2016), and those sounds accelerate their metamorphosis (Stanley et al. 2015). Stanley et al. (2010, 2012) also found that five brachyuran crab species had shorter time-to-metamorphosis when exposed to local rocky reef soundscapes compared to silent controls in the laboratory and field.

Non-arthropod invertebrates: A variety of sessile invertebrates respond to the soundscapes of settlement habitat (e.g., corals, oysters, mussels, ascidians), though it is not understood how they perceive and distinguish among soundscapes. An in-situ study of the larvae of a reef building coral (*Orbicella faveolate*) showed that larvae move towards underwater speakers broadcasting coral-reef noise but moved in random directions when no sound was broadcasted (Vermeij et al. 2010). Lillis et al. (2016, 2018) observed higher settlement of two species of coral larvae in in-situ settlement chambers in response to high quality (i.e., high coral and fish diversity and abundance) coral-reef soundscapes compared to low quality reefs. Larvae of the oyster *Crassotrea virginica* settled at higher densities when to oyster-reef soundscapes than soft-bottom soundscapes or no-sound treatments in the laboratory and in in-situ chambers where oyster larvae were exposed to oyster reefs compared to nearby sandy bottom (Lillis et al. 2013). Field experiments also demonstrated greater settlement of free-swimming oyster larvae on larval collectors exposed to oyster-reef soundscapes compared to silent collectors (Lillis et al.
Common biofouling taxa such as mussels (Wilkens et al. 2012) and ascidians (McDonald et al. 2014, Stanley et al. 2016) also exhibit faster settlement and metamorphosis when exposed to anthropogenic sound from ship engines and generators.

Other marine larvae avoid certain soundscapes. Simpson et al. (2011) found that two common pelagic taxa and some nocturnally active species actively avoided light traps with broadcasted reef soundscapes and were more common in control light traps. Parmentier et al. (2015) found that some fish taxa avoided certain habitat soundscapes. Indeed, there is mounting evidence that suggests that habitat degradation alters the properties of soundscapes and, in turn, larval attraction. Piercy et al. (2014) compared coral-reef soundscapes across a quality gradient and found that higher quality coral reefs are louder, and their soundscapes may propagate farther. Butler et al. (2016) found that degraded backreef hardbottom soundscapes were significantly quieter than healthy hardbottom soundscapes. The degradation of nursery habitats and their soundscapes may have consequences for the larvae that use sound to locate settlement habitat. Playbacks of pre-degradation coral-reef soundscapes were more attractive to fish larvae than post-degradation soundscapes (Gordon et al. 2018) and more coral larvae settle in response to high-quality reef soundscapes than to soundscapes of low-quality reefs (Lillis et al. 2016). In backreef hardbottom habitat, settlement of a variety of taxa to collectors in degraded habitat were lower than in loud, healthy habitats (Butler 2016). Given the important role that soundscapes play in larval settlement, alterations to soundscapes caused by habitat degradation may hamper recruitment and natural recovery.

SOUND AND SETTLEMENT IN BACKREEF HABITATS
In the Caribbean, most studies on underwater sound and larval recruitment have focused on coral-reef habitats (Vermeij et al. 2010, Staaterman et al. 2013, Lillis et al. 2016) until the recent work by Butler et al. (2016, 2017) on larval fish and invertebrate settlement in response to sounds from backreef habitats. The backreef habitats in south Florida are a mosaic of tropical habitats that provide important connectivity among life stages and energy transfer among habitats. Seagrass and mangroves are well known nurseries for coral-reef associated species (Nagelkerken et al. 2000, Heck et al. 2003). But backreef karst hardbottom habitats dominated by sponges are also crucial nursery habitats for many of the same reef-species and other ecologically and commercially important species such as spiny lobster, stone crab, and commercial sponges (Herrnkind et al. 1997). However, cyanobacteria blooms have caused mass die-offs of sponges in a portion of Florida Bay leaving large swaths of barren habitat with few sponges and associated fauna (Butler et al. 1995, Herrnkind et al. 1997, Stevely et al. 2011). “Healthy” hardbottom, with high sponge density and biomass, provides habitat for numerous soniferous organisms, particularly snapping shrimp. “Degraded” hardbottom, areas that experience mass sponge die-offs, contain little habitat for snapping shrimp and thus exhibit significantly lower spectra levels (sound power as a function of frequency) than healthy hardbottom (Butler et al. 2016). Restoration of the sponge community through sponge transplants restored the natural soundscape (see Fig. 2 in Butler et al. 2016), indicating that restoration of the sponge community promoted the return of the soniferous biological community (Butler et al. 2016).

Given that soundscapes are a strong potential settlement cue for a wide range of taxa it is not surprising that Butler (2016) found fewer larval settlers on silent collectors deployed in degraded hardbottom than those that were exposed to recordings of healthy soundscapes.
broadcasted at above natural levels. Also, fewer fish and invertebrate larvae settled in natural, degraded hardbottom sites compared to unaffected healthy hardbottom sites. However, restored sites, while producing similar soundscapes to healthy hardbottom, also had significantly lower settlement than healthy sites. These experiments indicate that healthy hardbottom soundscapes are a likely settlement cue for a broad range of taxa that settle in shallow hardbottom habitat, but that degradation of the sponge community has altered the settlement cue for large areas of potential nursery habitat. Alteration of the acoustic settlement cue could reduce settlement and recruitment of larval fish and invertebrates, further hindering recovery of these degraded areas. Reestablishing the sponge community may return the settlement cue, however, the results of these previous studies suggest that the range of the cue for these small restoration patches may be limited and preclude larval settlement from returning to pre-degradation levels.

The aim of my project was to gain a better understanding of fish and invertebrate larval response to hardbottom soundscapes. I hypothesized that since healthy habitats have higher larval settlement than degraded habitats, that broadcasted healthy soundscapes would likewise increase settlement over degraded soundscapes. I also hypothesized that settlement rates would vary as a function of the distance from the sound source. To test this, I first determined the maximum range of the acoustic cue. I then examined larval settlement at increasing distances from an underwater speaker broadcasting healthy soundscapes at natural levels, simulating a small, restored patch of habitat, compared to broadcasted degraded soundscapes.
METHODS

STUDY AREA

The studies were carried out in Florida Bay and nearshore waters just north of the middle Florida Keys (USA) where there is a mixture of seagrass meadows, sandy-mud bottom, mangrove islands, and sponge-dominated karst hardbottom (Fig. 1). These habitats vary considerably in their soundscape profiles. Healthy hardbottom produces soundscapes that contain high levels of high frequency sounds (>1000Hz) and large numbers of snapping shrimp snaps (Butler et al. 2016). In contrast, seagrass beds are far quieter than healthy hardbottom (Butler et al. 2016) and absorb sound (Wilson et al. 2013). Degraded hardbottom sites are significantly quieter than healthy sites with fewer snapping shrimp snaps and soundscape spectra similar to seagrass beds (see Fig. 2 in Butler et al. 2016). The present study takes advantage of the now quiet, barren hardbottom areas in a large region in the central Florida Keys affected by sponge die-offs, as a location in which I could broadcast experimental soundscapes with minimal interference from natural soundscapes.

TRANSMISSION LOSS

Modeling sound propagation in shallow waters, such as Florida Bay, is difficult (Urick 1983) and many of the necessary physical parameter estimates (e.g., bedrock depth and density) have not been made. Therefore, the easiest method to estimate transmission loss was with empirical measurements. In July 2018, I conducted a transmission loss experiment in degraded hardbottom to determine the approximate distance that an auditory cue from a playback device can be detected. I defined this point as the distance from the source where the signal can no
Figure 1. Map of sampling sites including the approximate extent of hardbottom habitat degraded by cyanobacteria blooms and sponge die-offs.

longer be distinguished from the background noise and the signal to noise ratio (SNR) is 0 dB. SNR is the ratio of the intensity of the signal ($I_S$) to the intensity of the background level ($I_N$), expressed in dB, and calculated as:

$$SNR = 10\log_{10}(I_S/I_N)$$

The detection threshold for animals is well above 0 dB SNR (Fish: Ladich 2013, Fay 2011, Chapman 1973; manatees: Gaspard et al. 2012; pinnipeds: Southall et al. 2000; cetaceans:
Branstetter et al. 2017). Therefore, within a few decibels, the SNR ratio can be considered as 0 dB for the practical purpose of determining the range of detection for marine larvae.

I deployed an omnidirectional underwater speaker (Lubell Labs 916H underwater loudspeaker; frequency response 200Hz – 20kHz, 180db re 1μPa output @ 1kHz), connected to a waterproof barrel containing a WAV player, an amplifier to drive the speaker, and a 12 V deep cycle battery to power the speaker and amplifier (Fig. 2). Then, I broadcasted pure tones of known frequency (100Hz, 500Hz, 1000Hz, 1500Hz, and 2000Hz) and amplitude (115dB re 1μPa) in degraded hardbottom habitat. Pure tones were chosen to represent a range of frequencies observed in natural hardbottom soundscapes without the variation in amplitude and frequency inherent to recordings of natural habitats. I recorded the tones using Aquarian Audio H2a omnidirectional hydrophones (Aquarian Audio Products: sensitivity -180dB re 1V/μPa [+/- 4dB 20Hz-4kHz]; flat frequency response 10 Hz – 100kHz), attached to Roland Edirol R-05 or R-07 solid-state WAV recorders (Roland Corporation, Japan; 48kHz; 16bit) contained in waterproof housings at increasing distances (1 m, 10 m, 25 m, 50 m, 100 m, 200 m, and 500 m) from the speaker (see Fig. 2D). The SNR was calculated for each distance as described above.

LARVAL RESPONSE TO SOUNDSCAPE AND DISTANCE

Soundscapes from healthy and degraded hardbottom habitats were broadcasted at sites within Florida Bay using the system described above to test the effects of soundscape type and distance from the source on larval settlement. Three pairs of sites (where in a pair of sites consists of one site where healthy hardbottom soundscapes were broadcast and one site where degraded hardbottom soundscapes were broadcast) were haphazardly chosen. All sites were at least 3 km apart and were within the ~500 km² area of Florida Bay affected by the sponge die-
offs, and thus represented separate sources of sound within a relatively quiet background environment. For each pair of sites, experiments were run twice: once during a full moon and
once during a new moon to capture the differences in larval settlement that are common between the two moon phases (Butler 2016). All larval collections were made between July and November 2019.

At each site, three artificial collectors were placed in opposing directions at four distances from the speaker: ~1 wavelength (10 m), mid-range (100 m), ~0 dB SNR (500 m), and out of range (1000 m, Fig. 3). Wavelength is proportional to frequency such that lower frequencies have longer wavelengths, therefore 10 m was chosen as the ~1 wavelength distance because it was the approximate length of the lowest frequency the speaker could produce given the speaker’s frequency response. Mid-range and ~0 dB SNR distances were selected based on the transmission loss experiment in hardbottom habitat. Collectors were made of frayed rope attached to a mesh back 50 cm x 100 cm in size tethered to concrete blocks and suspended in the water column by a surface buoy (Fig. 3). These collectors mimic the physical structure of hardbottom vegetation that many settling larvae use and have been successful in previous larval studies in the area (Butler 2016). Collectors were placed in healthy hardbottom habitat for six weeks prior to the start of the experiment to develop a biofilm and were shaken prior to the start of each trial to remove any larvae that may have settled between trials so that only larvae settling during the trials would be collected.

Prior to the start of the experiment, soundscapes from several haphazardly selected healthy and degraded sites were recorded at new and full moons using an omnidirectional hydrophone (described above). Recordings were only used for one trial to avoid pseudoreplication (Kroodsma 1989). Average root-mean-square sound pressure level over a 15 second clip was calculated for each recording and used to calculate the required voltage output for the speaker system in order to broadcast the recordings at approximately the same volume at
which they were originally recorded. Voltage output was matched by manipulating the volume of the WAV player containing the broadcasted recording prior to deployment of the speaker. The sound level of the recorded soundscapes used in the larval settlement ranged from 76-80 dB re 1 µPa for healthy soundscapes and 65-68 dB re 1 µPa for degraded soundscapes.

![Figure 3. Larval collector and experimental collector placement. Three artificial larval collectors, made of frayed rope attached to a mesh back with ¾ in PVC pipe top and bottom frame and tethered to a buoy and anchor made of cement blocks (A [Photo credit: Jack Butler] were deployed at four distances (10 m, 100 m, 500 m, 1000 m) from an omnidirectional underwater speaker system broadcasting either healthy or degraded hardbottom soundscapes.](image)

Trials were run for five nights: two nights before a full or new moon to two nights after the moon phase of interest. During each trial, a speaker was deployed at each site and broadcasted either a healthy or a degraded hardbottom soundscape at approximately the same volume as the original recording. Speakers were deployed each evening of the trial and retrieved
the following morning. Collectors were sampled the morning following the last night of the trial. Collectors were unclipped from the mooring and carefully moved into a mesh bag (1 mm$^2$ mesh) before being brought aboard a vessel where they were shaken to dislodge the larvae into the bag. Larval fish were separated and immediately euthanized by overdose with tricain methanosulfonate (MS-222) following an IACUC approved protocol (#19-026). Fish and invertebrate larvae were then preserved in 70% ethanol for quantification and identification to the lowest taxonomic level.

Larval community assemblage data were Hellinger transformed prior to analysis, visually represented with two-dimensional non-metric multidimensional scaling (nMDS) and compared using a non-parametric (permutational) multivariate analysis of variance (PERMANOVA). The Hellinger transformation was chosen because it does not give high weights to rare species, which occurred sporadically in this data set, and it makes the data more suitable for ordination techniques (Legendre and Gallagher 2001). PERMANOVA uses traditional analysis of variance experimental design extended to a matrix of pairwise distances with P-values obtained by permutation (Anderson 2001). PERMANOVA is a flexible multivariate test that allows any distance measure and is useful for community composition data (Anderson 2001). Moon phase, distance, and soundscape were fixed factors, and site treated as a random variable. Analyses were run using the vegan package (v2.5-6; Oksanen et al. 2019) in R 3.6.3 (R Core Team, 2020).
RESULTS

TRANSMISSION LOSS

The SNR decreased exponentially with distance and at 500 m was nearly undetectable (~3dB, Fig. 4). Because the recordings used in the larval settlement experiment (65-80dB re 1µPa) were quieter than the tones used in the transmission loss experiment (115dB re 1µPa), I fit a curve to the transmission loss results (Fig. 4) and calculated the expected 3dB SNR distance for the broadcasted soundscapes. Based on the curve, the estimated 3dB SNR distance for the broadcasted soundscapes ranges from approximately 490 m – 497 m for healthy soundscapes and 466m–474m for degraded soundscapes. Therefore, the distances used for collector placement are still appropriate for the sound levels of the broadcasted hardbottom recordings.

LARVAL RESPONSE TO SOUNDSCAPE AND DISTANCE

Over the course of the experiment 8,551 individual recruits of 41 different taxa were collected. Bivalves (50.9%, 4,353 individuals, 5 species) and crustaceans (35.3%, 3,017 individuals, 6 species) comprised the majority of the catch. Table 1 summarizes the total numbers of individuals and species collected by moon phase, soundscape, and distance. The larval assemblage differed between moon phases (PERMANOVA: $F_{1,141} = 6.225$, $R^2 = 0.043$, $p = 0.001$), soundscapes (PERMANOVA: $F_{1,141} = 1.863$, $R^2 = 0.013$, $p = 0.001$), but did not differ among distances (PERMANOVA, $F_{1,141} = 0.985$, $R^2 = 0.021$, $p = 0.085$). Overall, more larvae settled during full moon phases than new moons (full moon = 4,926 total individuals, new moon = 3,625 total individuals) and mean individuals per collector was 1.3 times higher during full moon than new moon (full moon = 68.4 ± 26.6 95% CI; new moon = 51.2 ± 13.7 95% CI).
Collectors exposed to healthy hardbottom soundscapes had greater overall settlement and a 1.4-fold increase in average settlement per collector (5,006 total individuals; 70.5 ± 28.4 95% CI) compared to collectors exposed to degraded soundscapes (3,545 total individuals; 49.9 ± 7.6 95% CI).

Figure 4. Signal to noise ratio (SNR) and regression line of pure tones broadcasted at maximum volume in degraded hardbottom calculated from hydrophone recordings taken at seven distances (1 m, 10 m, 25 m, 50 m, 100 m, 200 m, 500 m) from the playback device. At 500 m from the playback device, the signal is nearly undetectable from the background noise (SNR = 2.6dB).
Table 1. Total number of individuals and taxa in parentheses collected over the course of the experiment from artificial collectors deployed at 10 m, 100 m, 500 m, and 1000 m from an underwater speaker broadcasting healthy or degraded soundscapes during new and full moon.

<table>
<thead>
<tr>
<th></th>
<th>New Moon</th>
<th></th>
<th>Full Moon</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy</td>
<td>Degraded</td>
<td>Healthy</td>
<td>Degraded</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>10 m</td>
<td>479 (18)</td>
<td>488 (19)</td>
<td>605 (16)</td>
<td>487 (20)</td>
<td>2059</td>
<td>(41)</td>
</tr>
<tr>
<td>100 m</td>
<td>395 (18)</td>
<td>500 (19)</td>
<td>664 (20)</td>
<td>521 (24)</td>
<td>2080</td>
<td>(40)</td>
</tr>
<tr>
<td>500 m</td>
<td>310 (15)</td>
<td>416 (21)</td>
<td>533 (20)</td>
<td>371 (22)</td>
<td>1630</td>
<td>(39)</td>
</tr>
<tr>
<td>1000 m</td>
<td>637 (15)</td>
<td>400 (22)</td>
<td>1383 (18)</td>
<td>362 (20)</td>
<td>2782</td>
<td>(38)</td>
</tr>
<tr>
<td>Total</td>
<td>1821 (22)</td>
<td>1804 (29)</td>
<td>3185 (30)</td>
<td>1741 (35)</td>
<td>8551</td>
<td>(41)</td>
</tr>
</tbody>
</table>

There was no difference in the mean number of settlers between collectors at 10 m and 100 m from the speaker systems (57.2 ± 13.7 95% CI and 57.8 ± 16.4 95% CI) but larval settlement on collectors deployed at 10 m and 100 m was 1.3-fold higher than 500 m (45.6.6 ± 12.4 95% CI). Even more larvae settled on collectors furthest from the sound source (1000 m = 79.5 ± 55.5 95% CI) as compared to collectors placed at 10 m, 100 m, and 500 m. Given that the collectors at 1000 m were deployed at more than twice the range of the speaker systems, the high mean settlement at this distance was not due to the effects of the broadcasted soundscapes. However, the effects of distance on mean larval settlement were small when separated by moon phase and soundscape (Table 2, Fig 5).
Table 2. Mean individuals ± 95% confidence interval collected from artificial collectors deployed at 10 m, 100 m, 500 m, and 1000 m from an underwater speaker broadcasting healthy or degraded soundscapes during new and full moon

<table>
<thead>
<tr>
<th></th>
<th>New Moon</th>
<th></th>
<th>Full Moon</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy</td>
<td>Degraded</td>
<td>Healthy</td>
<td>Degraded</td>
</tr>
<tr>
<td>10 m</td>
<td>53.2 ± 27.5</td>
<td>54.2 ± 18.5</td>
<td>67.2 ± 47.8</td>
<td>54.1 ± 27.7</td>
</tr>
<tr>
<td>100 m</td>
<td>43.9 ± 15.5</td>
<td>55.6 ± 20.0</td>
<td>73.8 ± 54.6</td>
<td>57.9 ± 46.2</td>
</tr>
<tr>
<td>500 m</td>
<td>34.4 ± 29.4</td>
<td>52.0 ± 19.0</td>
<td>59.2 ± 34.6</td>
<td>41.2 ± 26.4</td>
</tr>
<tr>
<td>1000 m</td>
<td>79.6 ± 110.2</td>
<td>44.4 ± 10.6</td>
<td>153.7 ± 225.2</td>
<td>40.2 ± 17.9</td>
</tr>
</tbody>
</table>

The nMDS (Fig. 6, stress: 0.153, $r^2 = 0.993$) plot places each artificial collector on a two-dimensional ordination plane based on the larval assemblage. Fig. 6A-C illustrates the effects of moon phase, distance from the speaker system, soundscapes, and collection period (one sequential new and full moon phase) on the larval assemblage of individual collectors. While significant in the PERMANOVA model, the $R^2$ values for each of the fixed factors and the nMDS suggest that the factors account for a small portion of the total variance (Table 3). The nMDS suggests that site or sampling period, which are correlated as sites were moved after a full and new moon period, may account for some of the variance in larval assemblage.
Figure 5. Mean number of larvae and taxa per collector for collectors deployed 10 m, 100 m, 500 m, and 1000 m, from broadcasted healthy (closed circle) and degraded (open circle) soundscapes during new and full moon phases. All error bars are 95% confidence intervals. Dashed lines indicate collectors that lie outside the detectable sound range (1000 m).
Figure 6. Non-metric Multidimensional Scaling ordination of larval assemblage samples. Each point represents the larval assemblage found on an individual collector. (A) Collector points colored by the moon phase during collection (blue = full moon, red = new moon). (B) Shapes and colors indicate the distance of the collector from the playback device (red circle = 10 m, orange triangle = 100 m, blue square = 500 m, green diamond = 1000 m). (C) Collector points colored by collection period (red = July/August, green = October, blue = November) and shape indicates the broadcasted soundscape (circle = degraded hardbottom, square = healthy hardbottom). Each collection period includes one full and one new moon sampling session. (D) Plot of influential species vectors, lengths are scaled by their correlation so that strong predictors have longer arrows than weak predictors.
Table 3. PERMANOVA results testing the effects of moon phase, soundscape type, and distance from the underwater speaker on larval community assemblage.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
<th>R^2</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moon Phase</td>
<td>1</td>
<td>2.474</td>
<td>6.225</td>
<td>0.043</td>
<td>0.001</td>
</tr>
<tr>
<td>Soundscape</td>
<td>1</td>
<td>0.740</td>
<td>1.863</td>
<td>0.013</td>
<td>0.001</td>
</tr>
<tr>
<td>Distance</td>
<td>3</td>
<td>0.392</td>
<td>0.985</td>
<td>0.021</td>
<td>0.085</td>
</tr>
<tr>
<td>Moon Phase x Soundscape</td>
<td>1</td>
<td>0.461</td>
<td>1.159</td>
<td>0.008</td>
<td>0.106</td>
</tr>
<tr>
<td>Moon Phase x Distance</td>
<td>3</td>
<td>0.178</td>
<td>0.448</td>
<td>0.009</td>
<td>0.875</td>
</tr>
<tr>
<td>Soundscape x Distance</td>
<td>3</td>
<td>0.202</td>
<td>0.509</td>
<td>0.011</td>
<td>0.772</td>
</tr>
<tr>
<td>Moon Phase x Soundscape x Distance</td>
<td>3</td>
<td>0.273</td>
<td>0.688</td>
<td>0.014</td>
<td>0.400</td>
</tr>
<tr>
<td>Residual</td>
<td>126</td>
<td>0.397</td>
<td></td>
<td>0.880</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>141</td>
<td></td>
<td></td>
<td>1.000</td>
<td></td>
</tr>
</tbody>
</table>

Inspection of the larval collector data revealed that two collectors of the 35 deployed at 1000 m had extremely high settlement, as much as 7 times greater than the collector with the next highest number of individuals at 1000 m. At both of the collectors *Lima* clams had large settlement events, up to 4.2 times greater than collector with the next highest number of clams (1012 and 356 vs 196 individuals). Removing these two collectors removed differences in mean settlement between 500 m and 1000 m (500 m: 45.6 ± 12.4 95% CI, 1000 m: 44.0 ± 8.9 95% CI) and effect sizes between the distances inside the range and both distances outside the range of the speaker were the same (1.3x). Removal of these collectors altered the effect of the soundscape treatment on mean settlement where healthy soundscapes had only slightly higher mean settlement than degraded soundscapes (1.1x; 53.3 ± 13.7 95% CI and 49.9 ± 7.6 95% CI,
respectively). The effect of moon phase on mean settlement was only slightly altered (1.2x; 46.7 ± 6.4 95% CI and 56.3 ± 11.2 95% CI).

Species richness did not vary among treatments (Fig. 5), but larval responses to moon phase, soundscape, and distance varied among taxa (Fig. 7, Table 4). Some taxa had greater settlement during new moon than full moon, such as the spiny lobster *Panulirus argus* (new moon: 0.8 ± 0.1 SE; full moon: 0.2 ± 0.1 SE) whereas others settled in higher numbers during full moon (e.g. *Lima* [new moon: 15.9 ± 5.3 SE; full moon: 31.9 ± 11.8 SE], *Paraclinus* [new moon: 0.4 ± 0.1 SE; full moon 0.9 ± 0.2 SE]). Several species settled in higher numbers on collectors within the range of the soundscapes (10 m and 100 m) compared to outside the range with a preference for healthy or degraded soundscapes. For example, the clam genus *Lima* settled in greater numbers during full moon and, with the two outliers at 1000 m removed, settlement was higher on collectors deployed at 10 m (37.9 ± 20.5 SE) and 100 m (26.4 ± 12.2 SE) from speakers broadcasting healthy soundscapes than on collectors outside the range (500 m: 14.4 ± 8.2 SE; 1000 m: 13.9 ± 6.6 SE [Fig. 7D]). The blenny genus *Paraclinus* was found in greater numbers during full moon and in greater numbers within the range of the speakers broadcasting degraded soundscapes than outside (Fig. 7F, Table 4). The shrimp *Palaemonetes* had opposing settlement patterns with higher settlement on degraded soundscape collectors during new moon and healthy soundscape collectors during full moon (Fig. 7G & H). Overall, 29 species had low (< 50 individuals) settlement with many absences and no discernable relationship to moon phase, type of broadcasted sound, or distance from sound source (Table 5).
Figure 7. Mean individuals of selected taxa per collector during new and full moon phases on collectors exposed to broadcasted healthy (closed circle) and degraded (open circle) soundscapes at 10 m, 100 m, 500 m, and 1000 m from the underwater speaker. All error bars are standard error. Selected species are shown: spiny lobster (*Panulirus argus* [A-B]), clam (*Lima* [C-D]) with two outliers at 1000 m removed, blenny (*Paraclinus* [E-F]), shrimp (*Palaemonetes* [G-H]). Dashed lines indicate collectors that lie outside the detectable sound range (1000 m).
Table 4. Total number of larvae per taxon with > 50 total settlers onto collectors deployed at 10 m, 100 m, 500 m, and 1000 m from an underwater speaker broadcasting healthy or degraded soundscapes during new and full moon.

<table>
<thead>
<tr>
<th>Species</th>
<th>Healthy 10m</th>
<th>Healthy 100m</th>
<th>Healthy 500m</th>
<th>Healthy 1000m</th>
<th>Degraded 10m</th>
<th>Degraded 100m</th>
<th>Degraded 500m</th>
<th>Degraded 1000m</th>
<th>Total Individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paraclinus sp.</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>9</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Bulla sp.</td>
<td>20</td>
<td>3</td>
<td>0</td>
<td>14</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Cerithium sp.</td>
<td>53</td>
<td>22</td>
<td>26</td>
<td>52</td>
<td>19</td>
<td>21</td>
<td>21</td>
<td>6</td>
<td>21</td>
</tr>
<tr>
<td>Echinolitorina sp.</td>
<td>27</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Turbo castanea</td>
<td>8</td>
<td>3</td>
<td>2</td>
<td>6</td>
<td>7</td>
<td>10</td>
<td>14</td>
<td>1</td>
<td>3</td>
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<tr>
<td>Nudibranch</td>
<td>4</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>3</td>
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<tr>
<td>Bivalves</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Lima sp.</td>
<td>124</td>
<td>162</td>
<td>137</td>
<td>376</td>
<td>90</td>
<td>111</td>
<td>46</td>
<td>67</td>
<td>341</td>
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<tr>
<td>Spondylus sp.</td>
<td>35</td>
<td>34</td>
<td>35</td>
<td>54</td>
<td>57</td>
<td>71</td>
<td>34</td>
<td>39</td>
<td>61</td>
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<tr>
<td>Portunus sayi</td>
<td>27</td>
<td>13</td>
<td>16</td>
<td>7</td>
<td>12</td>
<td>15</td>
<td>12</td>
<td>13</td>
<td>19</td>
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<td>Arthropods</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Panulirus argus</td>
<td>12</td>
<td>12</td>
<td>8</td>
<td>4</td>
<td>8</td>
<td>7</td>
<td>5</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Palaemonetes sp.</td>
<td>149</td>
<td>102</td>
<td>62</td>
<td>108</td>
<td>270</td>
<td>235</td>
<td>248</td>
<td>227</td>
<td>141</td>
</tr>
<tr>
<td>Annelid</td>
<td>16</td>
<td>13</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>5</td>
<td>6</td>
<td>5</td>
</tr>
</tbody>
</table>

New Moon

Full Moon
Table 5. Total number of larvae collected for taxa with < 50 settlers collected over the course of the experiment during new moon and full moon.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>New Moon</th>
<th>Full Moon</th>
<th>Total Individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fish</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abudefu saxatilis</td>
<td>19</td>
<td>13</td>
<td>32</td>
</tr>
<tr>
<td>Acanthus</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Haemulon</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Histrio histrio</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Opsanus</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Sygnathus</td>
<td>15</td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td>Unknown larval fish</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Gastropods</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Australium</td>
<td>0</td>
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<td>3</td>
</tr>
<tr>
<td>Cantharus</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Costoanachis</td>
<td>0</td>
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<td>4</td>
</tr>
<tr>
<td>Crepidula</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Diodora</td>
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<td>4</td>
</tr>
<tr>
<td>Modulus</td>
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<td>7</td>
<td>18</td>
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<tr>
<td>Nitidella</td>
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<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Pyrgospira</td>
<td>0</td>
<td>1</td>
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DISCUSSION

Underwater soundscapes are useful settlement cues for larval fish and invertebrates, but their usefulness depends on the range at which they can be detected (Radford et al. 2011b). In this study I examined larval responses to healthy and degraded hardbottom habitat soundscapes at increasing distances from an underwater speaker. I examined sound propagation from a point source, to determine the potential range of detection for marine larvae that may use sound to locate settlement habitat, then compared the larval assemblages that settled on collectors placed both within and outside of that radius.

Overall, larval settlement on collectors exposed to healthy soundscapes was higher than degraded soundscapes, although the response to soundscapes was species-specific. The PERMANOVA also indicated differences in larval assemblage between soundscapes. A few genera (e.g., Lima, Bulla) settled in greater numbers on collectors in response to healthy hardbottom soundscapes, in accord with an earlier study conducted in the same region (Butler 2016). Yet, other taxa (e.g., Paraclinus, Turbo) settled in greater numbers on collectors exposed to degraded soundscapes. These animals may prefer different nursery habitat types. Healthy hardbottom soundscapes are naturally louder and more complex (Butler et al. 2016) and the broadcasted recordings I deployed reflected that. Louder, more complex soundscapes could indicate a larger biological community and potentially more predators. Simpson et al. (2011) found taxa that had pelagic or nocturnally emergent lifestyles, avoided reef soundscapes. Unfortunately, the preferred nursery habitat for most of the more than 50 taxa observed in this study are unknown.
Larval assemblages did not differ among the different distances but overall, larval settlement was higher on collectors based at 10 m and 100 m from the speaker system compared to 500 m and 1000 m, once the two 1000 m outliers were removed. Soundscapes were played back at a natural level so the ~0dB SNR distance was slightly closer than the 500 m estimated from the sound propagation experiment, meaning settlement at this distance should be unaffected by the playback. As detection thresholds for most animals require SNRs upwards of 20 dB (Ladich 2013, Fay 2011, Chapman 1973), the radius for which healthy soundscapes can be detected is likely smaller than the estimated 0 dB SNR range. Collectors deployed at 1000 m, more than double the ~0 dB SNR range, were also well outside the range of the speaker system so settlement at those collectors should not have been affected by the soundscape treatment.

Increased larval settlement within the estimated detectable range of the speaker (up to 100 m) therefore indicates an effect of the broadcasted soundscape. High settlement at a few of the 1000 m collectors must therefore be due to other factors and not the broadcasted soundscapes. Degraded hardbottom may be so devoid of settlement habitat that the collectors, though outside the range of the broadcasted soundscapes, represented ideal settlement substrate and attracted more larvae.

The PERMANOVA indicated that larval assemblages differed between moon phases and settlement was higher during new moon overall. Notably, the most abundant fish and bivalve genera (Paraclinus, Lima, and Spondylus) in this study settled on collectors in greater numbers during full moon phases than new moon. Many animals use the lunar cycle to time release of larvae (Corals [Harrison et al. 1984, Brady et al 2016], Fish [Farmer et al. 2017]) and many larvae settle around specific moon phases (Fishes – bluehead wrasse [Victor 1986], damselfish and surgeonfish [Sponaugle and Cowen 1986], Decapods – crabs [Cannicci et al. 2019], lobster
Previous work in Florida Bay found higher settlement of marine larvae during new moon than full moon but with variation among species (Butler 2016).

Most of the species with 50 or more settlers followed a particular settlement pattern and these patterns varied widely between species. For example, the spiny lobster *P. argus* had higher settlement during new moon regardless of soundscape or collector distance. Patterns for distance were usually observed during a particular moon-phase when that species had the highest settlement. One such species is the clam genus *Lima*. During full moon, with the two outliers at 1000 m removed, *Lima* settlement was higher within the range of the healthy hardbottom soundscape (10 m, 100 m) than outside the range (500 m, 1000 m) or for broadcasted degraded soundscapes. Butler (2016) found greater numbers of *Lima* on collectors exposed to healthy hardbottom soundscapes compared to silent collectors during full moon. Both results suggest that *Lima* is responsive to habitat-associated sound cues, similar to other bivalves that have also demonstrated responses to sound cues (Lillis et al. 2013, Lillis et al. 2015).

Butler et al. (Butler, Anderson, and Butler unpublished data) established that there was no difference in the larval community between collectors exposed to degraded soundscapes and “silent” controls – collectors placed in degraded habitat near a silent mimic speaker. Since the degraded soundscapes were broadcast at natural volumes there should be little difference in soundscape between collectors inside and outside the range of the broadcast. However, a few genera were collected in greater numbers within the range of degraded soundscapes (e.g., *Paraclinus, Turbo*) than outside the range of the speaker system. This indicates that there is a difference between broadcasted degraded soundscapes and natural degraded soundscapes for at least some settlers. While I broadcasted soundscapes at ecologically-relevant natural volumes, there are artefacts of playback that alter the properties of the soundscapes. The speaker frequency
response drops off drastically below 200 Hz, so frequencies below 200 Hz in the hardbottom soundscape would not have been broadcast as loudly as they were recorded.

A few taxa had contradictory settlement patterns. For example, the shrimp, *Palaemonetes*, had high settlement during new moon on degraded soundscape collectors and on healthy soundscape collectors during full moon (Fig. 7I & J). I was unable to identify this genus to species and it is possible that more than one species was collected, each with contrasting settlement patterns. This highlights the need to look more deeply at the settlement preferences of *Palaemonetes* and other taxa with inconsistent settlement patterns.

Though not designed as a factor of interest in the experiment, site and collection date were correlated (one pair of sites were used for a consecutive full and new moon period, or one “collection period”) and could have affected observed settlement patterns. For many species, larval settlement varies both temporally and spatially (O’Beirn et al. 1996, Acosta et al. 1997, Martínez and Navarrete 2002, D’Alessandro et al. 2007). As site and date were intrinsically linked in this experiment, it was not possible to explore the individual contributions of these factors, but both are likely to contribute to the observed variation in larval settlement and community assemblage.

Halfway through the November full moon trial a cold front moved through the Florida Keys. Florida Bay is shallow and therefore rapidly warms or cools with changing air temperature and wind speed. The water temperature during this sampling session and the subsequent new moon sample was 5º-7º C cooler than the summer and October sampling periods (South Florida Natural Resources Center DataForEVER). Additionally, a cyanobacteria bloom developed in the area around my sites during the cold front, increasing chlorophyll content by ~13 µg/L, and remained well beyond the last sampling session (SFNRC DataForEver). Harmful algal blooms
can negatively affect the abundance of marine larvae present in the water column (Almeda et al. 2011) and blooms such as this are the cause for massive sponge mortality events. Algal blooms can alter dissolved oxygen concentrations (O’Boyle et al. 2016) and increase water viscosity (Seuront et al. 2006). Increased water viscosity, through changes in temperature or by blooms, can alter larval movement (Osse and van den Boogaart 1999, Hunt von Herbing 2002) and low dissolved oxygen can negatively affect larval settlement (Baker and Mann 1992). It is possible that such a bloom, in combination with the drop in temperature, could have affected larval settlement.

Some taxa were rare in the experiment (<50 total individuals). This low settlement could indicate low larval supply or the incorrect type of collector or habitat for that taxa. The collectors used in this experiment were designed to mimic benthic algae commonly used as settlement habitat by many mobile organisms within hardbottom habitat and therefore would not be ideal for organisms that prefer a different settlement substrate or for highly mobile organisms such as fish. While the collectors appeared to be adequate for capturing some benthic fish species such as the blenny, *Paraclinus*, other common backreef fishes were rarely caught (e.g., *Haemulon*, *Opsanus*) or not caught at all. Larval fish supply to the Florida Keys is lowest in late fall (D’Alessandro et al. 2007) which could also account for the low numbers of fish larvae that I observed in our summer-fall study period.

Estimating ecologically relevant levels of larval settlement requires estimating the number required to maintain or grow the population (Cowen et al. 2006). Recruitment to a population is often determined by resource availability (e.g., food and space), larval supply, and post-settlement processes (e.g. predation; Menge 2000, White and Caselle 2008). Most of the species captured in the collectors are understudied so their population and recruitment dynamics
are unknown. For *P. argus*, no correlations have been found with settling density and lagged age groups (Ehrhardt and Fitchett 2010). Habitat appears to be the factor limiting recruitment of this species to the population. This may be the case for many of the species found in the collectors since there is little suitable settlement habitat in degraded areas. If habitat is restored, even a small difference in larval settlement might translate to more adults because of the new available space allowing them to recruit to the adult population.

Given the small range of the broadcasted soundscapes that I measured, the soundscapes of small patches of healthy hardbottom habitat likely have a very limited range. This has implications for restoration efforts. Previous work in Florida Bay found greater larval settlement within healthy habitats than in degraded habitat (Butler 2016). But within small (25 m x 25 m) hardbottom patches, where the sponge community and soundscape had been restored, larval settlement did not match natural healthy hardbottom levels (Butler 2016). Though the soundscape had been restored within these small patches, they were a point source and therefore had a much smaller range of detection than the large area of healthy habitat (Piercy et al. 2014, Radford et al. 2011b). More larvae were potentially able to detect the sounds at greater distances and could explain why Butler (2016) did not see comparable settlement between collectors placed in small, restored patches and collectors in natural healthy hardbottom even though soundscapes had been restored. My results indicate that the range for a small point source of healthy hardbottom soundscape is several hundred meters but the effective detection range for larvae is perhaps only tens to a hundred meters.

While it is possible to restore soundscapes to natural levels, the small patch size of many restored sites may limit the range of the acoustic settlement cue, negating its effectiveness as a settlement cue for larvae and hindering recovery. Future work should examine what acoustic
range or level of acoustic connectivity between patches is necessary to restore the settling larval assemblage. A larger patch will have a larger detection range but several small patches within range of each other may extend that detection range with fewer resources. Patches close to large areas of healthy hardbottom may benefit from being near a potential source of larvae or colonizing animals, but the greater detection range may draw more larvae to the natural healthy habitat at the expense of the restoration patch. Restoration patch size, patch connectivity, and patch location are well studied concepts in other ecosystems (Shulz and Crone 2005, Fink et al. 2009, Morrison et al. 2010, Gittman et al. 2018) but have not yet been explored in hardbottom habitats.

This work adds to the growing body of literature investigating soundscapes as a settlement cue for marine larvae. However, my results were more nuanced than in other studies. Only a few species showed a strong response to broadcasted hard-bottom sounds and sound emanating from a point source at natural levels had a small range of attraction suggesting that habitat patches may not have the necessary range to draw in larvae over long distances. Small point sources, such as small restoration patches, may not be effective at restoring settlement to the same level as large areas of healthy habitat. However, sound is just one cue that larvae use to locate and choose settlement habitat and should be considered in conjunction with other settlement cues.
LITERATURE CITED


APPENDIX
TO: Dr. Mark Butler  
FROM: Dr. Stephen Beebe, IACUC Chair  
CC: Danielle Dady, Office of Research  
Re: IACUC protocol #19-026  
Date: December 12, 2019

Dear Dr. Butler,

This letter is to inform you that your protocol, "Larval fish and invertebrate responses to underwater sound; a field study" (IACUC protocol #19-026), has been APPROVED by the Old Dominion University IACUC and you are free to begin work on the project immediately. The decision date for this project is December 12, 2019.

Please remember that even minor changes to your protocol including personnel and procedural changes must be submitted to the IACUC using our amendment forms found under the ‘Forms and Templates’ section of IRBNet (www.irbnet.org). The amendment(s) must be submitted through IRBNet to the Office of Research in advance of implementing the change. Once these amendments are received and approved, you will be free to implement the changes. Any unapproved deviations in the protocol will be considered non-compliance and could result in a suspension and/or investigation.

In addition, this protocol will need to be renewed annually through the annual review process if you wish to continue your project past December 12, 2020.

Feel free to contact Danielle Dady in the Office of Research at 683-5451 to discuss any questions or concerns during your project.

Regards,

Stephen J. Beebe, Ph.D.  
Chair, Institutional Animal Care and Use Committee
VITA

Emily R. Anderson
Department of Biological Sciences
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H.B.S (June 2014) – Biology, Oregon State University, Department of Biology, University Honors College, Corvallis, OR

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2015 Marine Laboratory Intern, Sanibel-Captiva Conservation Foundation, Sanibel, Florida

2014 Field Research Intern, Heck Seagrass Ecology Lab, Dauphin Island Sea Lab

2014 Research Assistant, Hogan Population Ecology Lab, Texas A&M University – Corpus Christi

2013 Undergraduate Research Assistant, Hixon Marine Ecology Lab, Oregon State University

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Marine Conservation Scholarship, Women Diver’s Hall of Fame
Learner-Gray Marine Research Grant, American Museum of Natural History
Virginia S. Bagley Endowed Scholarship, Department of Biological Sciences, Old Dominion University
Harold G. and Vivian J. Marshall Scholarship, Department of Biological Science, Old Dominion University
Hollis Gear Award, American Academy of Underwater Sciences
Love of Learning Award, Phi Kapp Phi Honor Society
Biology Graduate Student Organization Travel Award, Old Dominion University
Student Support Award, Southern Association of Marine Laboratories
Fellowship of Women in Science Travel Award, Old Dominion University