Chapter 77
Soundscapes and Larval Settlement: Characterizing the Stimulus from a Larval Perspective

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Abstract There is growing evidence that underwater sounds serve as a cue for the larvae of marine organisms to locate suitable settlement habitats; however, the relevant spatiotemporal scales of variability in habitat-related sounds and how this variation scales with larval settlement processes remain largely uncharacterized, particularly in estuarine habitats. Here, we provide an overview of the approaches we have developed to characterize an estuarine soundscape as it relates to larval processes, and a conceptual framework is provided for how habitat-related sounds may influence larval settlement, using oyster reef soundscapes as an example.

Keywords Estuarine sounds • Acoustic cue • Drifting hydrophone • Bivalve settlement

1 Introduction

Successful recruitment of marine larvae is essential to replenishing populations and maintaining benthic communities (Roughgarden et al. 1988), but larvae are challenged with locating favorable settlement sites after a pelagic phase that can transport them vast distances (Kingsford et al. 2002). The underwater soundscape is a potentially rich source of sensory information for larval organisms during settlement and habitat selection because acoustic signals reflect the physical and biological characteristics of the environment (Montgomery et al. 2006; Cotter 2008; Radford et al. 2010). Compared with chemical and physical cues associated with the substrate on the scale of centimeters, habitat-related sound is a potentially broader scale signal that could facilitate larval encounter with a suitable settlement substrate over meters to kilometers (Montgomery et al. 2006; Lillis et al. 2013).

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A.N. Popper, A. Hawkins (eds.), *The Effects of Noise on Aquatic Life II*, Advances in Experimental Medicine and Biology 875,
DOI 10.1007/978-1-4939-2981-8_77

Recent studies have demonstrated that coral and rocky reef fish and crustaceans orient and settle in response to habitat-related sounds (e.g., Tolimieri et al. 2000; Jeffs et al. 2003; Simpson et al. 2005; Montgomery et al. 2006; Stanley et al. 2010, 2011), and our work has found a settlement response to oyster reef sounds by oyster larvae (Lillis et al. 2013; see Chapter 30 by Eggleston et al.). Despite this recent progress, the spatiotemporal scales of acoustic variation relevant to larval settlement have not been explored and information specific to the soundscape of estuaries, key settlement, and nursery habitats for a multitude of species is even more limited. Passive acoustic recordings have focused heavily on fish call identification for population and behavioral studies (e.g., Rountree et al. 2006; Locascio and Mann 2008), and previous habitat-related soundscape quantifications have largely consisted of very short-term (5-min), nonsimultaneous recordings that are unlikely to capture the sonic variation to which larvae are exposed during their pelagic phase. Moreover, we are unaware of studies investigating estuarine soundscapes from the perspective of larval dispersal and settlement. Here we highlight several complementary approaches that survey the acoustic characteristics of oyster reef and off-reef habitats in Pamlico Sound, NC, across a variety of spatiotemporal scales that likely match larval dispersal and settlement processes of estuarine invertebrates.

2 Study System

Pamlico Sound is a vast lagoontype estuarine system in the southeastern United States that contains a variety of habitats (e.g., salt marsh, oyster reef, sea grass, soft bottom) that serve as nursery grounds and adult habitats for numerous estuarine-dependent fishes and invertebrates. Oyster reef habitats represent an important functional role in the estuary because they provide a structured biogenic habitat that offers shelter and feeding opportunities for a plethora of resident and transient species (Boudreaux et al. 2006). Because oyster reefs are productive areas that harbor many sound-producing organisms and are patchily distributed habitats sought out by the larvae of many obligate reef dwellers, they are an ideal system in which to investigate habitat-associated sounds and their role in larval settlement. To examine the oyster reef soundscape and compare reef with off-reef acoustic characteristics, we have acoustically sampled a network of ten subtidal oyster reserves located throughout Pamlico Sound (interreserve distances of 20–105 km) since 2010. Simultaneously, we used a reef dweller (oyster) and a nonreef dweller (clam) as study organisms in laboratory and field experiments to examine larval settlement responses to estuarine soundscapes and to investigate the role of habitat-related sound to the larval settlement process (see Chapter 30 by Eggleston et al.).

3 Soundscape Characterization

Previous characterizations of spatiotemporal scales of variation in habitat-associated sounds have involved relatively short-term measurements, such as 2- to 5-min recordings at multiple locations at different times to compare sites or habitat types

(Kennedy et al. 2010; Radford et al. 2010; McWilliam and Hawkins 2013) or 5-min recordings at a single location every hour over a day in different seasons to assess temporal acoustic patterns (Radford et al. 2008). Three such studies have been performed to describe the acoustic patterns of temperate coastal habitats in New Zealand (Radford et al. 2008, 2010), and one has quantified spatial variation in coral reef sound profiles (Kennedy et al. 2010). Our data for Pamlico Sound, however, suggest that the use of a few minutes of data to represent a habitat type or a single day to represent a lunar phase is likely inadequate to characterize the spatiotemporal variation in acoustic spectra of seascapes. During initial acoustic sampling events in Pamlico Sound in June 2010, multiple recordings were made concurrently within the same oyster reserve but at different locations along the reserve boundary (100–400 m apart). These data demonstrated high acoustic variability in both space and time at relatively small scales. For example, over several hours at a single reserve, two hydrophones placed ~400 m apart measured root-mean-square sound pressure levels that varied substantially, with differences of up to 15 dB (Fig. 77.1a). Comparing the power spectral densities for the two stations at two 5-min periods during the

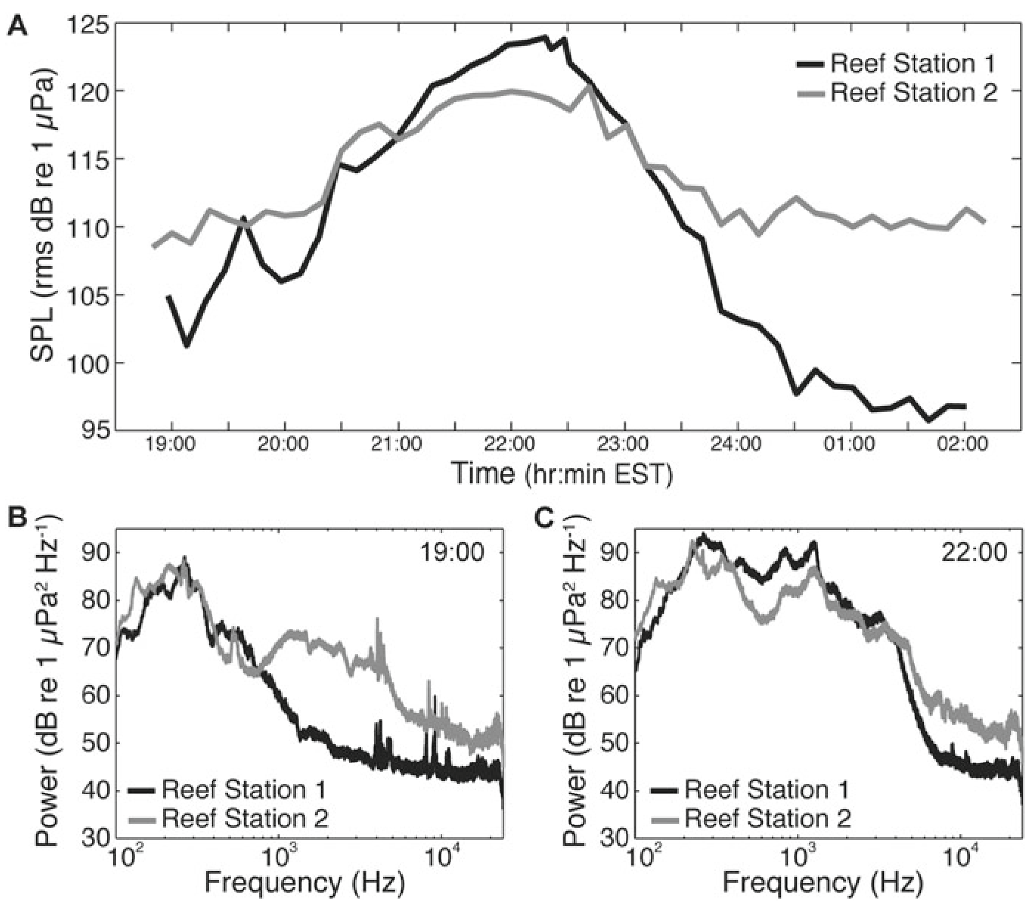

Fig. 77.1 (**a**) Comparison of broadband root-mean-square (rms) sound pressure levels (SPLs) measured overnight on 12 June 2010 at two recording stations at the Clam Shoal oyster reserve. The rms sound level was calculated for 1-min samples every 10 min. Power spectral densities at reef stations for a 5-min sample at 1900 hours (**b**) and a 5-min sample at 2200 hours (**c**). Power spectral density was estimated via Welch's method (Hamming window, 1-s averages with 50% overlap)

deployment (Fig. 77.1b, c) show substantially different spectra. From these initial data, it is clear that making relatively short-term recordings is inadequate to characterize variation in habitat-specific sounds (variation that is likely important to dispersing larvae that might use sound as a settlement cue) and that dramatically different conclusions could be drawn about the soundscape depending on the timing and location of sampling. Based on these results, we added several long- and short-term acoustic-sampling approaches to the overnight stationary recordings (described in Sections 3.1, 3.2, and 3.3) to better characterize the oyster reef and off-reef soundscapes at spatiotemporal scales relevant to oyster larval dispersal and settlement.

3.1 Stationary Hydrophone Habitat-Associated Sound Comparison

In the summer and fall 2010, passive sound recordings were made of subtidal oyster reefs and nearby soft-bottom habitats in Pamlico Sound. Hydrophones were deployed before dusk and positioned ~0.5 m from the seafloor. Hydrophone recording systems consisted of a calibrated omnidirectional SQ-26-08 hydrophone (flat-frequency response 0.03–30 kHz, sensitivity −169 dB re 1 V/μPa; Sensor Technology), and an M-Audio Microtrack II digital acoustic recorder (48-kHz sampling rate, 24 bit) with an external battery pack contained in a surface float. The recorder gain was set at the minimum level during all sampling, and the recorders were calibrated by recording pure-tone sine waves of multiple frequencies produced by a signal generator (Simpson Electric Function Generator 420) and comparing the measured root-mean-square voltage to the derived value (Au and Hastings 2008).

Oyster reefs and nearby soft-bottom habitats (~2–3 km from the oyster reefs) were acoustically sampled simultaneously each month for dusk and nighttime periods around the new moon (±3 days) at three sites spanning the length axis of the estuary. The data indicate that subtidal oyster reef habitats in Pamlico Sound consistently have distinct acoustic spectra, generally composed of significantly more sound in the ~2- to 20-kHz invertebrate-dominated frequency range, compared with the nearby off-reef soft-bottom habitats (Lillis et al. 2014). Based on spectral analysis and comparison with characterized vocalizations of fish common to the area (Sprague et al. 2000), the predominant biological sound sources within oyster reef environments were snapping shrimp (*Alpheus heterochaelis*) and fishes such as the oyster toad fish (*Opsanus tau*), weakfish (*Cynoscion regalis*), and Atlantic croaker (*Micropogonias undulates*). Off-reef areas were generally quieter and devoid of the loud snapping shrimp sounds but often had several-hour-long periods of very loud fish calls, likely due to sciaenid fish spawning events common in Pamlico Sound (Luczkovich et al. 1999, 2008). Unsurprisingly, there was variation in the sound levels among reef sites and within reefs over time, most likely due to differences in sound-producing animal distribution and abundance patterns, with variation in snapping shrimp activity appearing to drive much of the intersite differences. These habitat-specific differences in acoustic characteristics suggest that snapping shrimp are an extremely important soniferous species, with a distribution that contributes substantially to the estuarine soundscape. More information about the general ecology, environmental tolerances, and life history of snapping shrimp would be extremely valuable to the growing field of underwater soundscape ecology.

3.2 Oyster Reef Sound-Propagation Measurements

To investigate the sound emanating from oyster reefs, acoustic surveys were conducted at several oyster reserve sites in Pamlico Sound in 2010 and 2011. Ambient underwater sound was recorded at increasing distances from the oyster reserves in September 2010 and again in June 2011. Two hydrophone recording systems (as described in Section 3.1) were used for each sound-propagation survey; one was placed in the middle of an oyster reserve area and held stationary for the duration of the survey, while a second unit was used to make ~10-min recordings at various distances from the reserve (100, 250, 500, 1,000, 1,500, and 2,000 m). The water depth remained largely homogeneous (3–4 m) over this distance away from the reserve, and the bottom type was consistently sandy mud. The direction of the recording transect at each reserve was chosen based on the bathymetry of the surrounding area to best allow for measurements at distances up to 2 km.

Waveforms from these acoustic surveys were visually inspected using Audacity software to remove transient anthropogenic noise such as boat motors, and simultaneous recordings (from a stationary on-reef station and a given off-reef station) were cut to be the same length, leaving between 4 and 6 min of recording to analyze for each distance. Examinations of the power spectra for the recordings at increasing distances from the oyster reserves revealed that sound levels, particularly in the >2-kHz frequency range, decreased quickly away from the reef and were typically diminished by 15–20 dB within the first 500 m. Although certain reefs were quieter than others, as found in the reef/off-reef comparison, the patterns of sound propagation away from oyster reserves showed a consistent pattern at a variety of spatiotemporal scales, such as among sites across Pamlico Sound (interreserve distances of up to 100 km) and in different years and times of year. These data confirm that the elevated sound levels and frequencies associated with oyster reef habitat have high site fidelity and suggest that habitat-related sound is a good candidate cue to signal close proximity to the desired settlement substrate for reef dwellers.

3.3 Spatiotemporal Variation in the Soundscape Measured via Surface Drifter

To complement the stationary hydrophone acoustic data and to obtain higher spatial resolution measurements with a particular relevance to the planktonic larval phase, we conducted an acoustic survey of oyster reserves using drifting hydrophones.

Drifting acoustic recorders were deployed in August 2011 and again in August and September 2012 at six reserve sites to continuously measure the small-scale changes in sound because an oyster reef habitat is crossed by a passive surface drifter. These experiments were meant to simulate what a planktonic larva might experience as it moves toward and across a reef environment. Drifter units consisted of a free-floating barrel containing an M-audio Microtrack recorder, battery pack, and GPS unit and a calibrated omnidirectional hydrophone (Sensor Technology SQ-26-08 or High Tech, Inc., HTI-96) suspended 0.5 m from the surface. For each drifter trial, two units were deployed ~500 to 1,000 m away from the reserve boundary in the opposite direction of the current. The drifters were released at upstream locations intended to produce a drift that crossed through the oyster reserve. After drifter deployment, the boat was moved an adequate distance so as not to interfere with the recordings while still maintaining visual contact and the motor was shut off. Drifters were observed and collected once they had traveled ~500 m off the downstream edge of an oyster reserve.

Drifters further demonstrated the elevated sound levels and higher frequencies that dispersing larvae might experience as they approach and cross oyster reef habitats (see Fig. 77.2 for an example drifter spectrogram). A very strong reef sound signal was observed at most reserves; however, there were two reefs that were much quieter and with fewer snapping shrimp sounds, and these sites showed a much weaker "reef sound" signal during the drifts (A. Lillis, unpublished data). The drifter data are especially useful in developing a conceptual model of how reef sound could function as a settlement cue, and, in turn, informing laboratory experimental treatments to examine the larval responses to relevant levels of acoustic stimuli (Lillis et al. 2013; see Chapter 30 by Eggleston et al.).

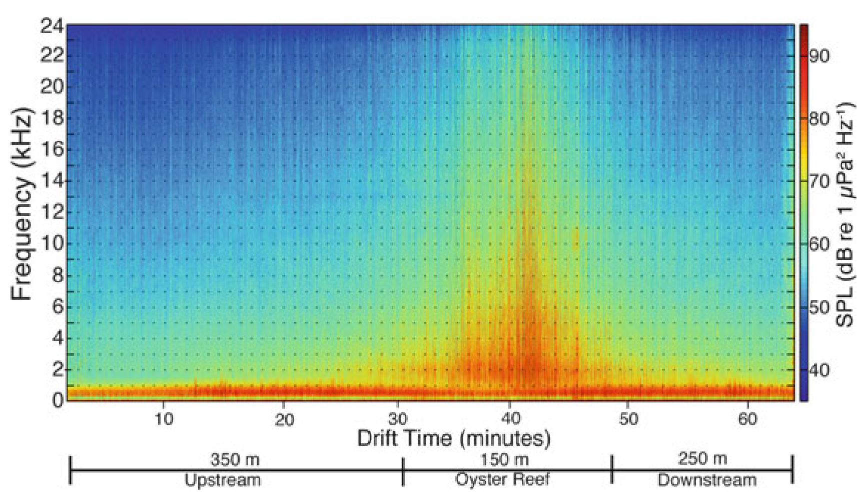

Fig. 77.2 Spectrogram of hydrophone drift across the West Bay oyster reserve (Hamming window, 1-s window with 25% overlap). Upstream, oyster reef, and downstream drift distances are indicated

4 Conceptual Model for Sound as a Settlement Cue

Integrating the results of the soundscape characterization and information of the larval life history and behaviors of our study species, we developed a conceptual framework for how spatiotemporal variability in habitat-related sounds may scale with larval dispersal and settlement to influence settlement outcomes. This conceptual model provides the mechanistic support for how sound could function as a settlement cue and serves as a useful guide for generating hypotheses and determining the relevant acoustic levels for testing them. For example, the results of the stationary soundscape characterization approaches suggest that oyster reefs have, in general, consistently distinct sound characteristics that are highly localized (i.e., a good indicator of close proximity to settlement habitat). The drifting hydrophone surveys, in turn, refined the spatiotemporal scales over which the soundscape can vary during the transport of a planktonic larva, with high-frequency (>2-kHz) constituents increasing substantially as the drifter approached the oyster reef and decreasing as it passed (Fig. 77.2). Given that late-stage oyster larvae can sink or swim at speeds between 0.12 and 0.3 m/min (Hidu and Haskin 1978), we predict that if oyster larvae respond to the oyster reef-associated sounds, they could reach the bottom from the surface (average water depth in Pamlico Sound is 4 m) in far less than the 30 min it took our drifter to cross a relatively small reef. Thus, larvae should have adequate time to respond to reef-associated sound characteristics by moving toward the bottom. After a substrate encounter, oyster larvae are expected to explore the substrate, selecting settlement habitat based on texture, chemical cues, and, potentially, acoustic characteristics. In contrast, we predict that clam larvae move to the bottom in the absence of reef sound, explore the substrate, and settle using substrate (chemical and physical) cues. Our conceptual model demonstrates how sound could play an important role in larval settlement for a weakly swimming larva. Although we illustrate the concept of sound as a settlement cue using oyster and clam larvae (our study organisms), it could be applied to larvae of other reef- and non-reef-dwelling organisms.

5 Conclusions

This work highlights the importance of extensive acoustic sampling over a broad range of spatial and temporal scales to compare habitat-related sounds and better understand the acoustic stimuli to which dispersing larvae may be exposed. We continue to conduct long-term recording time series at reef and off-reef sites to be able to better inform our laboratory and field settlement experiments and, subsequently, apply the results of laboratory and field experiments to the natural environment. This coupling of soundscape characterization and larval ecology shows great promise in improving our understanding of when and where sound may be important in recruitment processes and how anthropogenic noise or the degradation of attractive soundscapes might interfere with these critical ecological processes.

Acknowledgments Funding for this work was provided by National Science Foundation Grants OCE-1234688 and DDIG-1210292 and grants to Ashlee Lillis from the PADI Foundation, the American Academy of Underwater Sciences (AAUS), and the National Shellfisheries Association. We thank G. Plaia, B. Puckett, R. Dunn, K. Pierson, J. Peters, H. Eggleston, and B. Gericke for field assistance.

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