Final Report for the Award:
Ocean Life Institute

Can squid really hear?

Behavior, directional responses and hearing diversity in cephalopods

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This was a project built upon the initial novel data on squid hearing acquired by the PI. These data demonstrated that squid physiologically respond to sound. This was the strongest evidence yet that these keystone animals can hear, and this sensory modality may be found in more taxa (i.e., invertebrates) than previously considered. The broad aim of the proposed work was to examine the functional use sound by cephalopods and its broader relevance as a sensory cue in their sensory ecology. The work sought to broaden the potential use of sound through tests to determine if cephalopods behaviorally respond, and whether this sensory modality occurs in other species.

The specific goals of this project included:

Aim 1: Examine the function and use of sound by squid.

(a) Behaviorally evaluate the frequency range and sensitivity to which squid respond, to assess if squid can detect and perceive sounds.

(b) Examine cephalopod directional sensitivity, a primary function of hearing.

Aim 2: Address the broader relevance of cephalopod hearing by comparatively investigating the acoustic physiological response range and sensitivity of poorly understood cephalopod species with very distinct habitat and ecological characteristics.

Results

We consider this work a huge success and are grateful for the support. We addressed and exceeded all the objectives of this proposed work, including addressing sound use in additional ecologically relevant cephalopod species (the veined squid, *Loligo forbesi* and *Sepia officinalis*, the common cuttlefish), quantified the behavioral responses in two cephalopod species (*Doryteuthis pealeii*, the longfin squid and *S. officinalis*), and examined and found evidence supporting the hypothesis that adult cephalopods directional respond to sound. These are all key components of functional sound use by cephalopods and potentially other marine invertebrates. Since the inception of this project we have published a scientific paper and two book chapters describing the hearing abilities of two key prey species (squid and sand lance) (Mooney et al. 2012; Strobel and Mooney 2012; Samson et al. 2013). We also have several other papers that are close to submission and several other data sets at various stages of analyses.

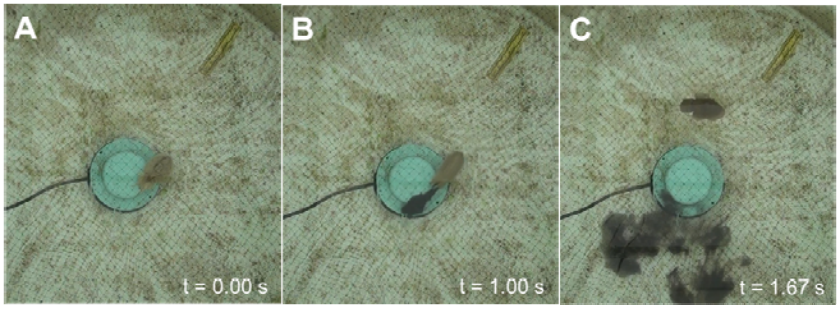

Figure 1. Examples of behavioral responses to sound. These frames are extracted from one test and illustrate how different behavioral responses can be combined. A: Cuttlefish at rest in the experimental tank before the sound stimulus. The median arms are dark and are hold backward over the head. B: Jetting and inking. C: Big color change (darkening) and fast fin movements resulting in a displacement of the animal after having jetted away.

The most exciting results showed that squid and cuttlefish behaviorally respond to sound (Fig. 1). These are the first data clearly defining the frequency range and sound levels that generate behavioral responses in marine invertebrates and indicate that squid and other cephalopods can use sound (predator, prey or navigation cues) in their natural environment and may be impacted by human-produced ocean noise.

To address these questions we developed a protocol for quantifying the potential behaviors cuttlefish and squid may elicit when they hear sound signals. This protocol will likely be applied to other species. Both species tested here exhibited a range of behaviors including inking, jetting, color change, fin movements and startle responses (Fig 1 and 2). This is novel work that not only provides behavioral sensitivity of cephalopods but also allows us to describe effective "loudness" curves. Loudness is the perception of sounds across different frequencies and sound levels (i.e., is a sound loud or quiet).

We also showed that animals habituate to repeated sound stimuli. There are few examples of habituation in any marine invertebrate. These results suggest that animals use sound (through rapid evasion-like responses) but can also filter out unimportant stimuli. The habituation studies also showed animals move directionally away from sound (escape-like responses) with the initial sound exposure. Based upon these results, a second experiment to explicitly test directional responses was also conducted. These data are being analyzed.

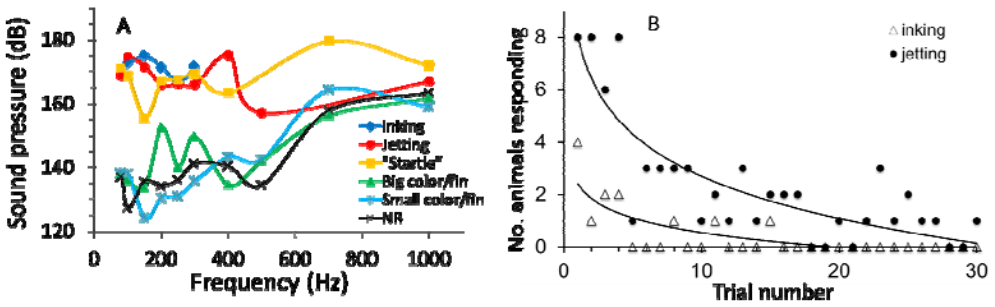

Figure 2. (A) Mean received sound levels in sound pressure and the behavioral responses they elicited for each tested frequency. Only the highest scoring behaviors for each sound test are represented here. At lower frequencies (below 500 Hz), the escape responses (jetting, inking and big color change) were elicited at higher sound levels. Above 500 Hz a relatively high sound level was needed to induce a response. (B) Number of animals responding across trial number, showing habituation (occurrence incidence decreased as number of exposures increased). These data were collected using a 3-s 200 Hz (165 dB) tone presented every minute for 30 consecutive trials. The occurrence of both response types (inking and jetting) decreased logarithmically.

We also completed a field project addressing the hearing of 10 veined squid in the Azores, Portugal. This was the first hearing test in a large squid species and the first physiological field measures in any squid species. All animals heard well with frequency ranges and response levels similar to the MA local longfin squid. The results suggest that hearing is conserved, at least among Loligiinid squids and perhaps among cephalopods and other marine invertebrates. The successful methods support further studies in other squid species. It also initiated a new collaboration between WHOI and Pedro Afonso, Research Scientist at the Univ. of the Azores, Portugal. This collaboration is already leading us to new projects.

Determining sound use, through tests of behavior and addressing uninvestigated species, is a substantial step toward defining the biological relevance of sounds and defining hearing in the ocean (still a controversial subject for marine invertebrates). Based upon this award, my lab has now shown sound-induced physiological and behavioral response in two squid and one cuttlefish species. They differ in their habitat and ecological characteristics. This work can be applied to ecological studies such as responses to predator signals but also the impacts of noise. These are exciting new results for a sensory modality poorly explored or understood in an ecological key taxonomic group. This work has been used to leverage and fund additional proposals. These data are currently being analyzed for publication and are being used to support larger proposals to investigate cephalopod sound use.

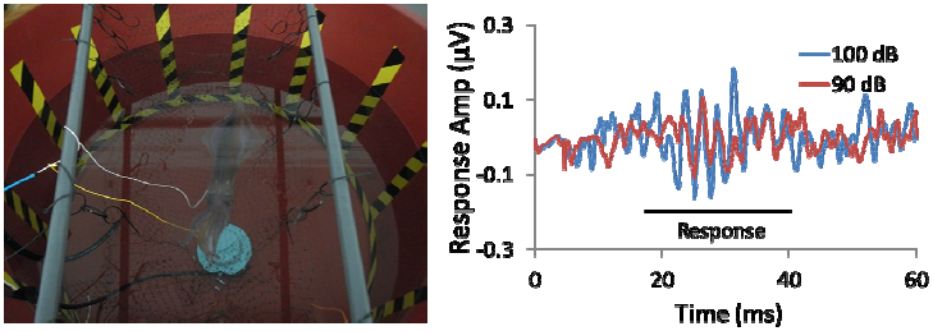

Figure 3. (Left) Squid during physiological hearing tests. Speaker (blue) is below the squid. It sits on a hammock of nylon mesh. Two electrodes are inserted into the animal. (Right) Physiological responses to a 200 Hz tone at two sound levels; recorded from squid Lf12_2013. These are only the second physiological hearing responses for squid species but supports the idea that multiple species of squid can hear.

Specific outcomes of this work

This work has been very successful. There have been three scientific peer-reviewed papers or book chapters as a result of the data collected and analyzed. Several more are in preparation. The results have been presented at multiple national and international conferences and venues, often as an invited talk. The work has received substantial attention from the public and news agencies, supporting overall "broader impacts." Capitalizing on the setup and field work, we leveraged several additional projects investigating (i) hearing and detection of predator sounds by sand lance, (ii) tag attachment methods for large, veined squid, (iii) swimming and respiratory fluid dynamics of the large, veined squid, and (iv) ocean acidification impacts to squid. Finally, the work is contributing to several larger NSF proposals to support future research. Scientific papers produced include:

(Mooney et al. 2012; Strobel and Mooney 2012; Samson et al. 2013)

Submitted Related Proposals

1) NSF, Biological Oceanography - Coral Chorus: The role of soundscapes in coral reef larval recruitment and biodiversity. T. Aran Mooney, Joel Llopiz and Amy Apprill (in review)
2) NSF, Biological Oceanography – Sound Science: The Vital but Variable Role of Sound Detection in Pelagic Invertebrate Ecology
3) NSF, Biological Oceanography - Functional Use of Sound in Cephalopods: Avoidance Responses, Navigation and Acoustic Ecology. T. Aran Mooney, Roger T. Hanlon and Jean G. Boal
4) NSF, Integrative Organismal Systems: Functional Sound Detection by a Basal Auditory System. T Aran Mooney, Roger T. Hanlon, Jean R. Boal
5) NSF, Integrative Organismal Systems: Invertebrate Adapted Hearing: Sound Detection by a Basal Auditory System. T Aran Mooney, Roger T. Hanlon, Jean R. Boal, Trevor Wardhill, and Richard R. Fay.
6) National Science Foundation, 2012-2015, Ocean Acidification: Examining impacts on squid paralarval development, behavior, and survival. T. Aran Mooney
7) WHOI's Innovative Technology Program - The I-TAG (Invertebrate Tag): Coordinated measures of invertebrate behavior and their natural environment using novel "eco-sensors". T. Aran Mooney, Tom Hurst, Alex Shorter, and Kakani Katija
8) Joint Industry Program: Are Squid Impacted by Airguns? Hearing, anatomy, and swimming behavior. T. Aran Mooney
9) Schmidt Marine Science and Technology Foundation: The I-TAG (Invertebrate Tag): Coordinated measures of invertebrate behavior and their natural environment using novel "eco-sensors". T. Aran Mooney, Kakani Katija and Pedro Afonso
10) NOAA SeaGrant: Squid Behavioral Responses to Sound. T Aran Mooney and Roger T. Hanlon.

Upcoming – NSF, Biological Oceanography, NSF, Integrative Organismal Systems, NSF Oceanographic Technology and Interdisciplinary Coordination.

Scientific Papers (published)

Mooney T, Hanlon RT, Christensen-Dalsgaard J, Madsen PT, Ketten DR, Nachtigall PE (2012a) The potential for sound sensitivity in cephalopods. Popper, AN, Hawkins, AD (ed) The Effects of Noise on Aquatic Life. Springer Science+Business Media, LLC, New York, pp 125-128

Samson J, Mooney TA, Hanlon RT, Guskerloo S (2013) Reviewing cephalopod behavioral to sound. Popper, A, A, H (ed) The Effects of Noise on Aquatic Life. Springer Science+Business Media, LLC, New York, pp in press

Strobel S, Mooney TA (2012) Detection of low-frequency tones and whale predator sounds by the American sand lance *Ammodytes americanus*. J Fish Biol 81:1646–1664

Scientific Papers (in preparation)

Samson, J, Mooney, TA, Hanlon, RT and Guskerloo, S. Behavioral responses and habituation to sound stimuli in the common cuttlefish, *Sepia officinalis*. In prep to coauthors.

Scharr A, Mooney TA, Schweizer F, Ketten DR (2012) Aminoglycoside induced damage in the statocyst of squid, *Doryteuthis pealeii*. In prep to coauthors.

Mooney, TA, Afonso, P and Katija, K. Hearing the largest Loligiinid squid, *Loligo forbesi*. In analyis.

Mooney, TA. Cephalopod directional movement in response to acoustic signals. In analysis.

Mooney, TA. Squid behavioral responses to sound stimuli vary in frequency and amplitude. In analysis.

Conference and Invited Research Presentations:

1. Kaplan, M*, **Mooney, TA,** McCorkle, D, Cohen, A. 2013. Adverse effects of ocean acidification on early development of squid (*Doryteuthis pealeii*). PLoS ONE 8(5): e63714. doi:10.1371/journal.pone.0063714.
2. **Mooney, TA**, Hanlon, RT, Christensen-Dalsgaard, J, Madsen, PT, Ketten, DR, and Nachtigall, PE. The hearing of the longfin squid (*Loligo pealeii*) and sensitivity to low frequency noise. 2nd International Meeting on "The Effects of Noise on Aquatic Life." Cork, Ireland, Aug 15-20, 2010.
3. **Mooney, TA**, Hanlon, RT and Streets, A*. Physiological and behavioral responses to sound in the longfin squid (*Loligo pealeii*). Ocean Sciences Meeting. Salt Lake City, UT, February 20-14, 2012.
4. **Mooney, TA,** Ketten, DR, Nachtigall, PE, Williams, CR, and Matassa, K. Using auditory evoked potentials to examine hearing loss in aquatic animals: From marine mammals to squid. 34th Meeting of the Association for Research in Otolaryngology. Baltimore, MD, February 19-23, 2011.
5. **Mooney, TA.** Marine mammal hearing and auditory evoked potentials. Department of Veterinary Medicine. National Chiayi University. Taiwan. December 16, 2011.
6. **Mooney, TA.** Eco-Physiology, bioacoustics and sensory ecology of marine animals. Department of Biology. National Cheng Kung University. Taiwan. December 15, 2011.
7. **Mooney, TA.** Sound sensitivity in cephalopods: Hearing, predator detection and ocean noise. University of North Carolina, Wilmington. September 30, 2011.
8. Samson, J**, **Mooney, TA**, Hanlon, R, and Guskerloo, S. Cephalopod behavioral responses and potential for habituation to acoustic stimuli. 3rd International Conference on the Effects of Noise on Aquatic Life. August 11-16, 2013. Budapest, Hungary.

9. Kaplan, M** and **Mooney, TA** Adverse effects of elevated CO_2 concentrations on squid (*Doryteuthis pealeii*) development and early life. Society for Integrative and Comparative Biology, San Francisco, CA, January 3-7, 2013.
10. Samson, JE**, **Mooney, TA**, Gussekloo, SWS, Hanlon, RT. Behavioral responses to sound stimuli in cuttlefish (*Sepia officinalis*). Society for Integrative and Comparative Biology, San Francisco, CA, January 3-7, 2013.
11. Kaplan, M* and **Mooney, TA**. Effects of elevated CO_2 conditions on paralarval longfin squid (*Loligo pealeii*) development and early life. Ocean Sciences Meeting. Salt Lake City, UT, Feb 2012.
12. Strobel, SM* and **Mooney, TA**. Sand lance detection of low frequency tones and humpback whale megapclicks. 19th Biennial Conference on the Biology of Marine Mammals. Tampa, FL. November 27-December 2, 2011.
13. Kaplan, M* and **Mooney, TA**. Impact of elevated CO2 conditions on paralarval longfin squid (*Loligo pealeii*) development and early life. WHOI Biology Department Summer Student Seminar. Woods Hole, MA. August 11, 2011.
14. Sharr, A* and **Mooney, TA**. Aminoglycoside-induced hair cell damage in the statocyst of squid (*Loligo pealeii*). MBL Undergraduate Research Symposium. Woods Hole, MA. August 18, 2011.
15. Streets, A* and **Mooney, TA**. Squid behavioral reactions to low frequency sound. MBL Undergraduate Research Symposium. Woods Hole, MA. August 18, 2011.