

Behavioral Responses to Underwater Sound in the Small Benthic Octopus *Octopus ocellatus*

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Abstract:

To explore behavioral responses to sound and the detectable frequency range of *Octopus ocellatus*, responses of the octopus to 120 dB rms sound stimuli of various frequencies were observed. The octopus did not respond at 200-1000 Hz, but clearly responded at 50-150 Hz by showing lengthened respiratory activities. Their respiratory activity was frequently suppressed for more than 5 sec after the sound stimulation, and the longest respiratory suppression reached 55.6 sec, while the mean respiratory activity length without sound stimulation was 1.23 sec. In the case of long-lasting respiratory suppression, they retracted the basal parts of their eyes simultaneously. This suggests that underwater sound may play an important role in the life of the octopus, possibly to detect predators.

Classification: Fishery and Bioacoustics

Keyword: cephalopod, hearing, octopus, particle motion

1. INTRODUCTION

There have been various attempts to control and modify fish behavior by emitting sound¹⁾. In fisheries, sound has been applied to drive fishes into nets, and attracting fishes using sound has been tried²⁻⁴⁾. It is also possible to keep fish away from a certain area (e.g., a water intake opening of a power plant) using sound⁵⁻⁹⁾. Moreover, the strong ability of associate learning in fish allows conditioning procedures to be applied for fish aggregations using sound in aquaculture¹⁰⁾. The sense organs and central nervous systems of cephalopods are developed equivalently to those of fishes¹¹⁾¹²⁾, so it is possible that cephalopod behavior

can be modified and controlled by artificially emitted stimuli in the same way as in fish¹⁾. Light has been successfully applied to increase squid catches in commercial fisheries, but the control and modification of cephalopod behavior by sound has received little attention. One of the reasons for this lack of attention to sound is that until 1990 it had been uncertain whether cephalopods have a sense for detecting sound.

In 1960, Hubbard¹³⁾ reported that octopus behavior was not modified by the presence of sound waves, a conclusion based on his classical conditioning experiments to train the octopus to associate sound with food. After this, cephalopods have been considered as

lacking sensitivity to sound¹⁴). In 1985, Moynihan¹⁵ argued that the "deafness" of coleoid cephalopods could be a consequence of their adaptation to avoiding damage from cetacean's stunnings, which can debilitate prey. In 1990¹⁶, it was shown by applying a classical conditioning procedure that 1) cephalopods were sensitive to the kinetic sound component (i.e., acoustic particle motion) which vibrates the whole animal, but not to the pressure component (i.e., sound pressure); 2) that it was possible to train cephalopods to associate sound with electric shocks; and 3) that the signal-to-noise ratio of the perception threshold of cuttlefish in the most sensitive range was comparable to that of cod. Since this study¹⁶, however, there have been no available studies about sound detection in cephalopods except a study about epidermal lines (i.e., lateral line analogue)¹⁷.

In the sound detection by cephalopods, most fields remain open; e.g., receptor(s), detectable frequency range, influences on behavior, development and function(s). The purposes of the present study were to investigate the detectable frequency range and behavioral responses to sound in the small benthic octopus, *Octopus ocellatus*. A previous study revealed that cephalopods are sensitive to low frequencies between 1 and 100 Hz¹⁶. This study focused on the higher frequencies above 100 Hz, rather than infrasound, to explore the upper limit of the detectable frequency range. As an indicator of responses to sound stimuli, respiratory activities, which were successfully applied not only to fishes¹⁸ but also to cephalopods¹⁶, were used. For cephalopods, the respiratory activities appear as mantle muscle movements, so that respiratory activities of the octopus were measured by recording the mantle muscle movements using an electromyograph. The animals examined were not trained to associate sound with unconditioned stimulus (e.g., electric shocks) in order that relatively "natural" responses to sound could be observed.

2. MATERIALS AND METHODS

Four specimens of *Octopus ocellatus* (mean \pm SD, body weight = 17.3 \pm 3.9 g) were used for the experiment. The animals were captured by hand on an intertidal sandy flat at Futtsu, Tokyo Bay, in June 2005. The animals were kept individually in a closed tank system at about 20°C in the laboratory of Tokyo University of Marine Science and Technology, Tokyo, Japan, for at least three days prior to the experiments.

A transparent plastic tank (180 \times 330 \times 230 mm) placed on a 5 mm-thick rubber seat was used as an experimental tank. Three vertical walls of the tank were sealed with white paper to conceal the experimenter from an experimental animal, while the wall opposite the experimenter was left transparent in order to monitor the animal using a video camera. Each experimental animal was put in a soft nylon net and placed in the experimental tank. The net was slightly bigger than the animals to allow them to breath and jet, but not to change their position¹⁹. Electrodes connected to an electromyograph (EMG; T-1202; FUKUDA, Tokyo, Japan) were attached to the net. Mantle muscle movements of the experimental animals were recorded by EMG via these electrodes. Although the electrodes were not attached to the muscle of the animal, the electrodes were extremely close to the animal, enough so to obtain electrical potentials produced by the mantle muscle movements. The EMG profiles were validated to indicate mantle muscle movements by comparing these profiles with visual observations on the video monitor.

To project sound, an oscillator (FG 273A; KENWOOD, Tokyo, Japan) was used. The signals were amplified by a power amplifier (AA6200; TOYODA, Osaka, Japan) and projected from an air speaker (C250L24s; FOSTER, Tokyo, Japan) fixed at 25 cm above the water surface. Air speakers have been successfully applied to investigate hearing abilities of fishes because, in comparison with an immersed sound

projector, the use of an air speaker reduces local water movement²⁰. The sound pressure level and frequency was measured in advance using a hydrophone (TC4013-4; RESON, Slangerup, Denmark) in the position where an experimental animal was to be set. An amplifier (VP1000; RESON, Slangerup, Denmark) was used to amplify the underwater sound detected by the hydrophone. The sound pressure was approximately 120 dB rms (dB re $1\mu\text{Pa}$) at each frequency (50, 100, 150, 200 and 1000 Hz).

Each test was initiated by a pre-stimulus EMG recording of 15 sec followed by a sound stimulus period of 7 sec and a post-stimulus EMG recording. Control tests were also conducted without sound projection. For each frequency and control, five tests were conducted for each individual, except for 100 Hz in two out of four individuals (BW = 13 and 21 g, four tests each). The animals sometimes showed a phase of hyperventilation and jetting in spite of an absence of stimulation, probably in order to escape from the net; in such cases, testing was halted until the animal relaxed¹⁶.

In order to indicate the magnitude of response to sound stimuli, respiratory cycle lengths were measured based on profiles of EMG records (**Fig. 1**) as the lengths between the upper or lower peaks, and a respiratory suppression ratio (SR) was calculated as described by Fay¹⁸. Fay defined the SR as the ratio of respiratory activity length to the length of an arbitrary unit minus the length expected for no respiratory activity. Since it was difficult to determine the length expected for no respiratory activity in the EMG profiles, the respiratory activity length was quantified in this study as the sum of the respiratory cycle length. Consequently, the SR in this study was redefined as $B/(A + B)$, where B was the sum of the 1st-3rd respiratory cycle lengths when the respiratory activity at the beginning of the stimulation was considered as the 1st, and A was the sum of the three respiratory cycle lengths immediately before the 1st respiratory cycle (Fig. 1b). As controls, B was the sum of the 1st-3rd respiratory cycle lengths when the respiratory cycle at 15 sec after the beginning of the EMG recording was considered as the 1st, and A was the sum of the three

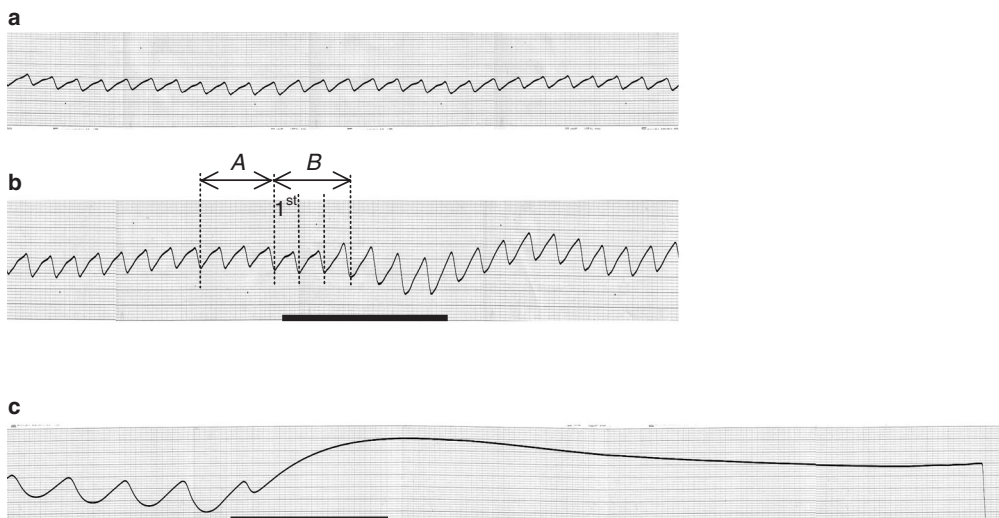


Fig. 1 EMG profiles of mantle muscle movements of *Octopus ocellatus* without sound projection (a) and with sound projection at 150 Hz (b) and 50 Hz (c). The 1st respiratory cycle at the beginning of the stimulation was indicated as 1st. A is the sum of the three respiratory cycle lengths immediately before the 1st respiratory cycle, and B is the sum of the 1st-3rd respiratory cycle lengths. The black bars indicate sound stimulus periods for 7 sec.

respiratory cycle lengths immediately before the 1st respiratory cycle. The SR thus varied between about 1 and 0.5, indicating complete respiratory suppression and stable respiratory activity (no respiratory suppression), respectively.

3. RESULTS

For the control tests without sound projection, the respiratory cycle length was about 1.23 ± 0.27 sec (mean \pm SD, $n = 120$; 6 respiratory cycles \times 5 control tests \times 4 individuals). For each individual, there was no significant difference between the mean SR of the control and 0.5, the hypothesized SR when the respiratory activity is stable (two-tailed t -test, $p > 0.1$; **Table 1**). This indicates that without stimulation, respiratory activities would not change in the tests conducted in this study. Therefore, provided that the SR of a test with sound projection was significantly different from that of the control, the difference would be considered to be the consequence of a response to the

sound projection. The SR is thus appropriate for indicating the responses of the octopus to sound.

Respiratory activity disturbances of *Octopus ocellatus* were observed when they were exposed to 50, 100 and 150 Hz sound (Fig. 1). For each individual, the SR at 50, 100 and 150 Hz were significantly greater than those of the control in three out of four animals (Mann-Whitney U -test, $p < 0.05$, **Fig. 2**). For one individual (BW = 15 g), clear respiratory disturbances were not found, and the SR was not significantly dif-

Table 1 Suppression Ratio (SR) of control tests (without sound projection) for the four individual *Octopus ocellatus* examined. n : number of tests; SD: standard deviation.

Body weight	n	mean	SD
15 g	5	0.502	0.0035
13 g	5	0.502	0.0076
20 g	5	0.506	0.0110
21 g	5	0.499	0.0037

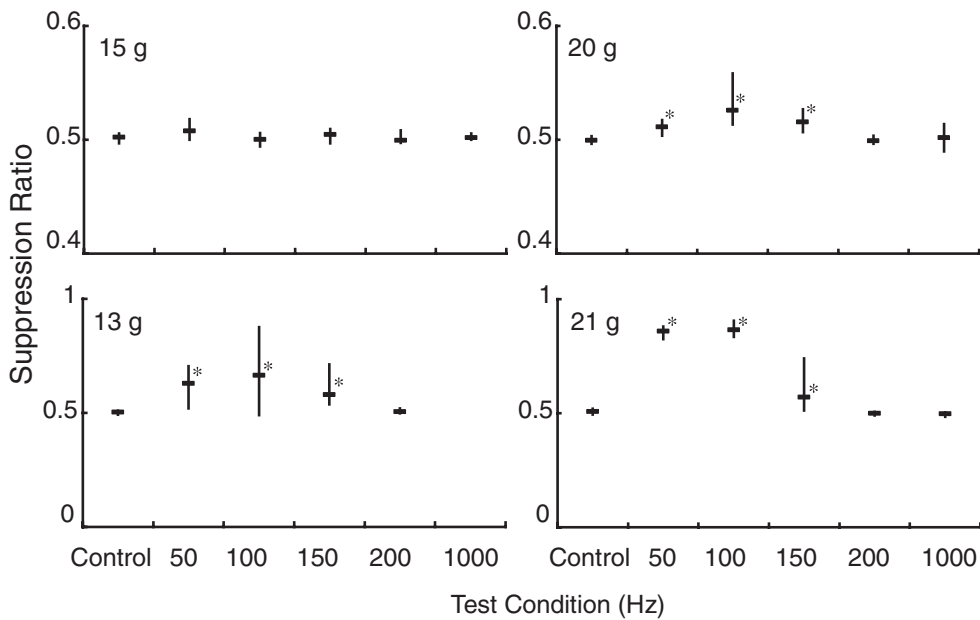


Fig. 2 Suppression Ratio (SR) of the four individual *Octopus ocellatus* examined. The X axis and the Y axis represent the test condition and SR, respectively. The horizontal bars indicate the mean SR, and the vertical bars indicate the ranges of SR. The body weight of each individual is indicated in the upper left of each panel. Significant differences from the control ($p < 0.05$) are indicated by *.

ferent from those of the control at any frequencies (Mann-Whitney U -test, $p > 0.05$, Fig. 2). In the 200 and 1000 Hz tests for all the individuals examined, no distinct disturbance in respiratory activities was observed, and the SRs were not significantly different from those of the controls (Mann-Whitney U -test, $p > 0.05$, Fig. 2). These results indicate that *O. ocellatus* responded to 50, 100 and 150 Hz sound at 120 dB, but did not to 200 and 1000 Hz sound. The behavioral responses of the octopus to sound appeared as lengthened respiratory activities because the SR at certain sound stimuli was greater than those of the control (Fig. 2).

Two of the three individuals (BW = 13 and 21 g) which clearly responded to sound suppressed their respiratory activities against the 50, 100 and 150 Hz sound (Fig. 1c). The respiratory suppression of one of those two individuals (BW = 13 g) lasted for more than five sec in three out of five tests at both 50 and 100 Hz, and the longest respiratory suppression reached 27.4 sec (at 100 Hz). The other individual (BW = 21 g) showed respiratory suppression that lasted for more than 10 sec in all tests at both 50 and 100 Hz and in one out of five tests at 150 Hz; the longest respiratory suppression lasted for up to 55.6 sec (at

100 Hz). These two individuals retracted the basal parts of their eyes (Fig. 3) simultaneously with a long-lasting respiratory suppression whenever their behavior was monitored by video camera. One individual (BW = 20 g) did not show such long-lasting respiratory suppression (Fig. 1c), although it clearly responded to sound with lengthened respiratory activity (Fig. 2). Although all the animals examined sometimes showed a phase of hyperventilation and jetting in spite of the absence of stimulation, long-lasting respiratory suppressions and the retraction of the basal parts of the eyes were not observed without stimulation.

4. DISCUSSION and CONCLUSION

In the present study, *Octopus ocellatus* responded to 50, 100 and 150 Hz sound at 120 dB with lengthened respiratory activities, but not to 200 and 1000 Hz sound. However, one individual (BW = 15 g) did not show clear responses to sound. Moreover, in the other three individuals (BW = 13, 20 and 21 g), behavioral responses appeared in different ways as a slightly lengthened respiratory activity (Fig. 1b) and a longer-lasting respiratory suppression (Fig. 1c). This suggests that the behavioral responses of *O. ocellatus* to sound may depend on individuals and/or conditions such as

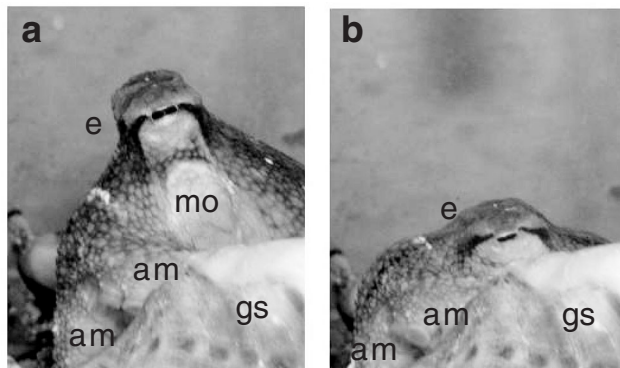


Fig. 3 Different positions of the basal part of the eyes of *Octopus ocellatus*: when the eyes are protruded (a) and when the eyes are retracted (b); e: eye; am: arm; mo: mantle opening; gs: gastropod shell as a shelter. The photos were taken in the rearing tank used to keep the octopus prior to the experiment.

stresses caused by handling.

It was reported that cuttlefish (*Sepia officinalis*), squid (*Loligo vulgaris*) and octopus (*Octopus vulgaris*) were sensitive to infrasound, even at 1 Hz (the lowest frequency examined), and that their sensitivity dropped off above 10 Hz up to 100 Hz (the highest frequency examined)¹⁶. This is consistent with the previous study that *O. ocellatus* in this study were less sensitive to high frequencies above 200 Hz. The detectable frequency range of *O. ocellatus* for 120 dB sound reached 150 Hz, and based on the acute infrasound sensitivity of cephalopods¹⁶, it probably extends into the infrasound range.

As for the receptor used to detect sound, the statocysts of cephalopods have been assumed to detect the kinetic sound component^{16,21}. The cephalopod statocyst is an inertial accelerometer, which contains a dense statolith attached to sensory hair cells²¹⁻²⁶. When a cephalopod is accelerated by the kinetic sound component, the dense statolith would lag behind, bending the sensory hair cells, and thus would stimulate these cells. It was reported that air-borne sound stimulated crustacean statocysts, which share a common basic structure with cephalopod statocysts²⁷. The statocysts of the octopus were thus possibly responsible for the observed responses to air-borne sound in this study.

Cuttlefish (*Sepia officinalis*) and octopus (*Octopus vulgaris*) responded to sound by respiratory suppression while squid (*Loligo vulgaris*) responded by jetting, and the differences were assumed to be associated with their way of life because *S. officinalis* and *O. vulgaris* are benthic species that initially freeze when threatened¹⁶. Respiratory suppressions were also observed in the benthic species, *O. ocellatus* (Fig. 1c), in this study. Their respiratory suppression lasted for up to 55.6 sec with retracting of the basal parts of the protruded eyes (Fig. 3). Freezing, which restricts the activity, would reduce the probability of detection and recognition by predators, and this trait of anti-predator

defense is generally found in many animals²⁸. It is reasonable to consider that the respiratory suppression of *O. ocellatus* in responding to sound is one of their defenses against predation. Moreover, retracting body parts that had been protruded would make them inconspicuous. Because being inconspicuous (*e.g.*, camouflaging) is a general trait of defense against predation in benthic cephalopods²⁹, retraction of the basal parts of the eyes would also be one of the defenses against predation. Considering that sound stimulation causes the octopus to exhibit specific behaviors that seemed to be related to defense against predation, it is likely that underwater sound would be one of the cues that inform the octopus of the presence of predators.

A great number of fishes make underwater sound by teeth stridulation and/or swim bladder vibration³⁰. The frequency ranges of such kinds of sound were extended below 100 Hz in many predatory fishes, including Serranidae, Lutjanidae, Carangidae and Gadidae³⁰. In addition, a swimming fish acts as a sound source with the dominant frequencies being between 100 and 500 Hz³¹. It is possible that these sounds may inform *O. ocellatus* of the presence of predatory fishes because the frequency ranges of the sounds produced by fishes frequently lap over into the detectable frequency range of *O. ocellatus*.

Because the animals examined in this study were not trained to associate sound with an unconditioned stimulus (*e.g.*, electric shocks), the behavioral responses that were observed should be similar to those found in nature. Based on the behavioral responses of *O. ocellatus* to sound in this study, it was clear that the behavior of this species of octopus was modified by the presence of underwater sound. Therefore, the modification of cephalopod behavior by sound emissions is possible, even though the ability to control their distribution to increase fisheries catches is still uncertain. The findings of the present study, however, indicate that future studies about the behavioral responses of

cephalopods to sound could open up possibilities for applying sound emissions to cephalopod fisheries and aquacultures.

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