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Are mussels able to distinguish underwater sounds? Assessment of the reactions of *Mytilus galloprovincialis* after exposure to lab-generated acoustic signals

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ABSTRACT

This study examined the effects of lab-generated acoustic signals on the behaviour and biochemistry of Mediterranean mussels (*Mytilus galloprovincialis*). The experiment was carried out in a tank equipped with a video-recording system using six groups of five mussels exposed to five acoustic treatments (each treatment was replicated three times) for 30 min. The acoustic signals, with a maximum sound pressure level of 150 dB rms re 1 μPa, differed in frequency range as follows: low (0.1–5 kHz), mid-low (5–10 kHz), mid (10–20 kHz), mid-high (20–40 kHz) and high (40–60 kHz).

The exposure to sweeps did not produce any significant changes in the mussels' behaviour. Conversely, the specimens exposed to the low frequency band treatment showed significantly higher values of the following biochemical stress parameters measured in their plasma and tissues: glucose, total proteins, total haemocyte number (THC), heat shock protein 70 (Hsp70) expression, and Acetylcholinesterase (AChE) activity.

The responses observed in the mussels exposed to low frequency sweeps enable us to suppose a biological and ecological role for this sound, which contains the main frequencies produced by both shipping traffic and the acoustic emissions of fish.

1. Introduction

Marine organisms use sounds during vital biological activities such as foraging, predator detection, mate attraction, and habitat selection (Au et al., 2000; Eggleston et al., 2016; Fay and Popper, 1998). Mainly in vertebrates, sound detection is considered to be a primary sensory modality, an important component of vital intraspecific interactions and a key way to detect the surrounding environment. The ability of marine invertebrates to detect and potentially use sound is poorly understood (Budelmann, 1992a,b). This is somewhat surprising given their relative abundance and central role in many marine ecosystems. Nevertheless, a growing body of literature suggests that marine invertebrates respond to sound in a variety of ways. Some authors have reported disturbance responses of crustaceans after exposure to anthropogenic sounds (Celi et al., 2013, 2015; Filiciotto et al., 2014, 2016) and behavioural changes in cephalopods to pure tone acoustic stimuli (Samson et al., 2014). There is a poor understanding of the frequencies and levels of sound that generate functional responses in most invertebrates, and thresholds have rarely been established. Only a limited amount of work has been carried out on the potential effects of noise on marine bivalves. It is known that oysters and clams develop statocyst structures in the pre-settlement stage (Cragg and Nott, 1977) and that, in bivalve molluscs, a putative hearing structure is represented by the abdominal sense organ (ASO), which is a mechanosensory receptor that is highly sensitive to mechanical waterborne vibrations (Zhadan, 2005).

Roberts et al. (2015) recently analyzed the behaviour of *Mytilus edulis* to anthropogenically-generated noise and in particular the valve closure as the indicator of reception and response.

Peng et al. (2016) showed that the exposure to anthropogenic sound (at ~80 and ~100 dB re 1 μPa) induced deeper digging and alteration in

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the O:N ratios and the expression of ten metabolism-related genes in the bivalve *Sinonovacula constricta*.

Due to their wide geographical distribution and ability to accumulate contaminants (Gatidou et al., 2010), mussels are commonly used as sentinel organisms in bio-monitoring studies and in the surveillance of the marine environment (Andral et al., 2004). In view of this, several methodologies for the assessment of the functional alterations that are rapidly induced by toxic contaminants have been developed (Vlahogianni et al., 2007), and some typical functional alterations related to the stress condition have been measured in mussels, particularly after their exposure to chemical pollution (Hellow and Law, 2003).

Recent studies have shown that molluscs exhibit an ancient form of a neuroendocrine response to stress that involves catecholamines and neuropeptides such as the adrenocorticotropic hormone (Ottaviani and Franceschi, 1996). The molecules of the stress response in molluscs are comparable to those in mammals: CRH, ACTH, cytokine-like molecules, biogenic amines (noradrenaline, adrenaline and dopamine) and cortisol-like molecules have been found in the haemocytes of gastropods and bivalves (Ottaviani and Franceschi, 1997; Ottaviani et al., 1998). In fact, it is well known in the literature on molluscs that different types of stress act on different biochemical parameters (Auffret et al., 2006; Haldane, 2002; Malagoli et al., 2007; Yao and Somero, 2013).

The Mediterranean mussel (*Mytilus galloprovincialis,* Lamark 1891) is one of the principal species, and is collectively widely distributed from temperate to subarctic coasts of both the Northern and Southern hemispheres. The Mediterranean mussel generally lives in infralittoral areas, from the top of the intertidal zone to depths of a few metres, attached to hard materials (rocks or piles) or substrates that are relatively movable (ropes) and to which it adheres by strong threads. It has a tendency to congregate and also forms numerous colonies.

In the present study, the Mediterranean mussel (*Mytilus galloprovincialis*) was exposed to lab-generated acoustic sweeps at different frequency ranges (from 0.1 to 60 kHz) in order to assess the behavioural patterns of mobility and the biochemical responses in plasma and tissue.

In particular, we addressed four questions during this research: "Are the mussels able to perceive underwater acoustic signals and respond to some specific acoustic frequency ranges? (2) Does the exposure to sounds induce any behavioural and biochemical changes in *M. galloprovincialis*? (3) If so, how do the behaviour and biochemistry change with the sound frequency levels? (4) What biological and ecological roles could underwater sounds play for this species?"

2. Materials and methods

2.1. Study species

Experiments were conducted between April and June 2015. Ninety mussels of 44.2 ± 6.99 g in weight (mean ± SD), 8.39 ± 0.49 cm in terms of the length of the maximum valve axes (mean ± SD), and 3.87 ± 0.29 cm with respect to the length of the minimum valve axes (mean ± SD) were used for the experiments. The animals were collected from a local farm and raised at the Marine and Coastal Environment Institute of the National Research Council (IAMC-CNR) in Messina, Italy.

2.2. Experimental set-up and protocol

Prior to the experiment, the mussel specimens were maintained in a 1.3 m^3 (1.3 m diameter and depth of 1.0 m) fibreglass tank. The experimental trials took place in a circular fibreglass tank that was identical in size to the holding tank. During the entire study period, the salinity was 38.23 ± 0.26‰ (mean ± SD), the temperature 18.62 ± 1.77 °C, and the dissolved oxygen 6–8 mg/l (mean ± SD, 7.41 ± 0.13). The tank was equipped with a flow-through system (with a complete renewal of the water each hour) of sea water. The photo and thermo periods were natural.

A total of 18 trials were carried out to test six experimental treatments from May to June 2015. After one month of acclimation period, the mussels were collected randomly, one by one, from the holding tank and assigned to an experimental trial. Five mussels were assigned to each trial. They were released into the centre of the experimental

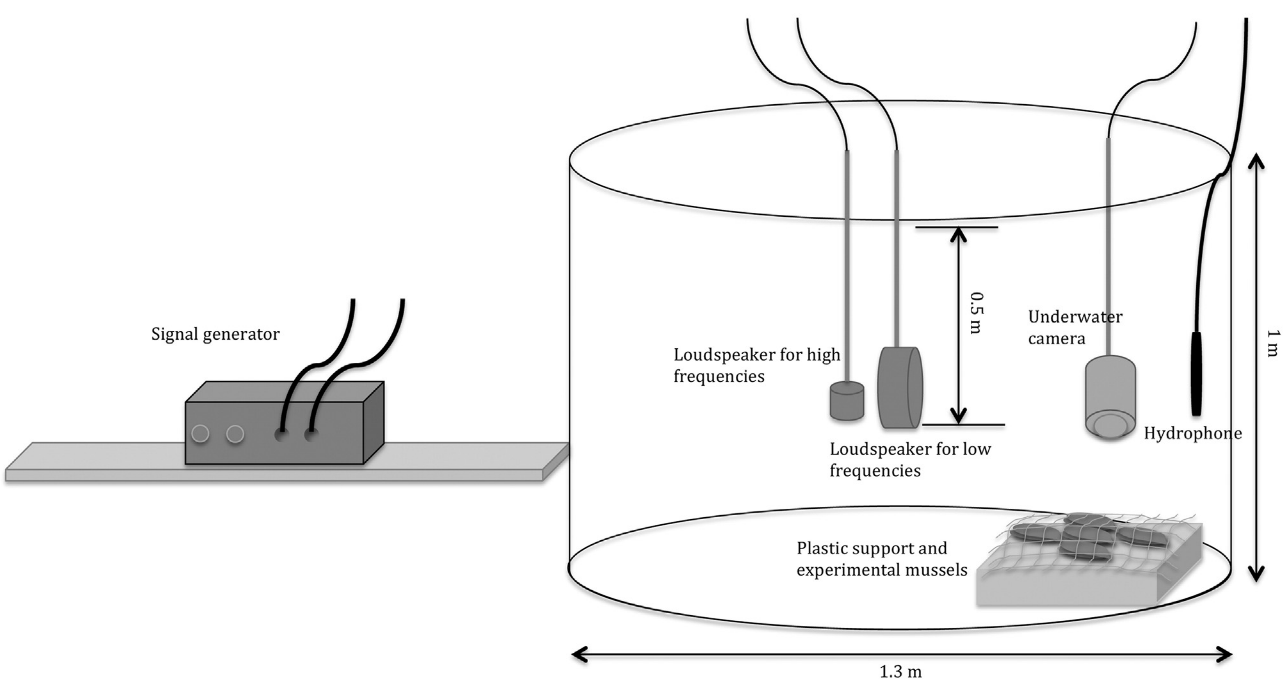

Fig. 1. Experimental tank set-up and audio/video equipment adopted in the study. The two loudspeakers were placed the centre of tank, at mid depth in water (0.5 m from surface and bottom, respectively) and both of them at the equal distance from the mussels (0.75 m). The hydrophone and the underwater camera were placed upon the mussels, respectively at about 0.45 m and 0.4 m from them.