TECHNICAL REPORT

Tank-based experiment and audio-video set-up to assess the impact of boat noise pollution on the common prawn (*Palaemon serratus*)

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Contents

1. INTRODUCTION AND BACKGROUND

In recent decades, anthropogenic activities, in particular shipping traffic, have led to increased sea noise pollution, causing background sea noise levels to change on a large scale (Ross, 2005; Hildebrand, 2009). Shipping traffic does not generally generate such intense noise, but the acoustic pollution it produces is constant over time, may affect large areas and could pose a serious hazard not only to individual animals, but also to entire populations (Weilgart 2007 ; Panigada et al. 2008 ; Clark et al. 2009 ; Slabbekoorn et al. 2010). Noise, can lead to alterations and other significant changes in marine habitats and animals, causing stress (Buscaino et al. 2010; Celi et al., 2013; 2015a, b; Filiciotto et al., 2013, 2014; McIntyre, 1995; Myrberg, 1990; Popper et al., 2004; Sarà et al., 2007; Wright et al., 2007; Wysocki et al., 2006), distractions (Hockey, 1970) and the masking of important sounds (Brumm and Slabbekoorn, 2005; Siemers and Schaub, 2011).

Despite their ecological and economic importance, or their value in terms of new natural products, the effects of noise on crustaceans have only recently been proposed (André et al., 2011; Celi et al., 2013; Filiciotto et al., 2013; Wale et al., 2013).

The common prawn (*Palaemon serratus*) is a sublittoral species of shrimp with a wide distribution, occurring along the northwestern Atlantic coast to the Mediterranean, Black Sea and Mauritian coast (Frasco et al., 2006). Even though this species provides an important link between trophic levels (Kelly et al., 2012), its bio-ecological responses to stress stimuli are still poorly understood. The hearing abilities of the common prawn have clearly been demonstrated in the work of Lovell et al. (Lovell et al., 2005) which, using ABR audiometry, has provided conclusive evidence of the sound detection of frequencies ranging from 100 to 3000 Hz in this invertebrate. The highest intensities produced by the shipping traffic usually fall within frequencies ranging from 0.1 to 1 kHz (McDonald et al. 2014).

Understanding the sound levels that generate behavioural responses in prawn and the physiological consequences of these responses would help us to evaluate the effects of boat noises on this species.

Despite of noise exposure changes the basal behaviour of many crustacean species, no specific data are available regarding the effects of boat noise on *P. serratus*.

1.1 Objectives

In the present technical report, we described the experimental protocol including the audio-video setting-up adopted in order to assess the behavioural changes of the common prawn (*Palaemon serratus*, Pennant 1777) after the short-term exposure to boat noises. Prawn specimens, in control tank-based experimental condition, were exposed to a random sequence of boat noises such as recreational boats, hydrofoil, fishing boat and ferry boat and, their locomotor patterns were studied.

2. EXPERIMENTAL PROTOCOL

The present study was conducted at the Institute for the Marine and Coastal Environment of the National Research Council (CNR-IAMC) of Capo Granitola (SW Sicily, Italy). About 60 prawns were captured along the coast of Capo Granitola using 600 mm-long traps with a 10 mm mesh and two opposing funnels of 50 mm in diameter (Demers and Reynolds, 2002; Scalici and Gilberti, 2005) baited with pieces of fish and shellfish. After capture, the prawns were transferred to a circular PVC tank (diameter: 2.35 m, depth: 1.5 m) for the acclimation period. The animals were fed defrosted fish and molluscs once a day during this housing period.

The experimental trials were performed in a square fibre-glass tank (diameter: 1.0 m, depth: 1.5 m) equipped with an independent flow-through seawater system from a common source (25 ± 3.7 l min−1; mean ± SD). The salinity was 36.4 ± 0.81 ppt (mean ± SD) and the temperature 18.61 ± 0.39 °C (mean ± SD) during the entire study period.

The experimental tank was also isolated from the floor by adding two sheets of open-cell neoprene between the floor and the tank. A shelter (a calcarenitic block with circular shapes) was placed on the bottom of the tank. To avoid disturbing the animals inside the experimental tank, a laboratory enclosure was placed 2 m from away from it and the equipment required for audio-video recording and projection was installed there.

Prior to starting the trials, the specimens were moved from the housing tank to the test tank using a net. In the experimental procedure, 48 prawns in total were used. After a 1 h habituation period, the trials consisted of 30 min of sound exposure for only the animals treated with the boat noise condition. The sound exposure consisted of the projection of noise resembling a marine area with high shipping traffic condition. This was achieved using a random sequence of noises from the following motorboats: five recreational boats, a hydrofoil, a fishing boat and a ferry boat.

Specimens assigned to the control group were not exposed to any stimuli other than the low-level noise of the experimental tank environment (control condition). In total, three trials in the boat noise condition and three in the control condition were performed. Eight specimens of *P. serratus,* represented by both females and males, were used in each trial.

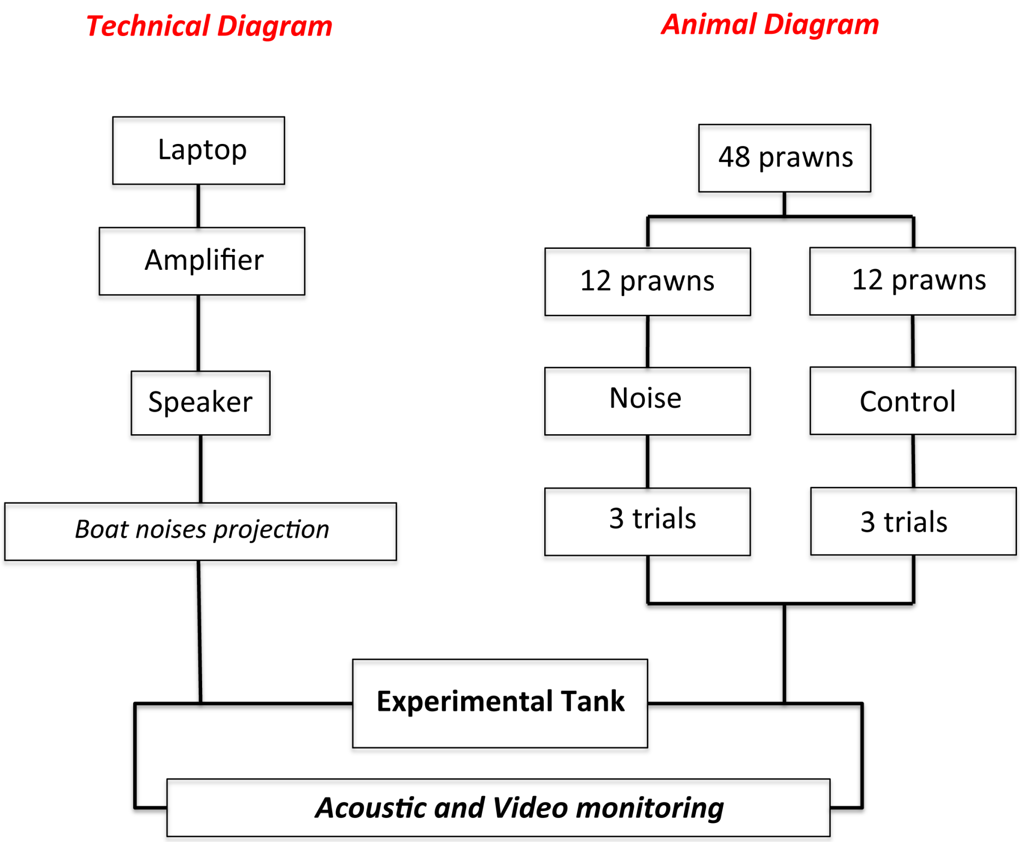

Figure 1. Schematic representation of the experimental protocol: technical and animal diagrams.

3. ACOUSTIC ACQUISITION AND PROJECTION SET-UP

To obtain acoustic recordings of boat noises, a calibrated hydrophone (model 8104, Bruel & Kjer, Nærum, Denmark) with a sensitivity of −205.6 dB re 1 V/µPa ± 4.0 dB in the 0.1-Hz to 80-kHz frequency band was used. The hydrophone was used with a preamplifier (VP1000, Reson, Slangerup, Denmark) with a 1-MHz bandwidth single-ended voltage that had a high-pass filter set at 10 Hz and a 32-dB gain. The equipment was connected to a digital acquisition card

(USGH416HB, Avisoft Bioacoustics, Berlin, Germany; set with no gain) managed by the Avisoft Recorder USGH software (Avisoft Bioacoustics). The signals were acquired at 300 kilo-samples s^{-1} at 16 bits and were analyzed by the Avisoft-SASLab Pro software (Avisoft Bioacoustics, Berlin, Germany).

During the entire experimental period, the seawater-recirculating flow was directly deployed beyond the tank water surface to prevent any bubbles, and no air pumps were used.

An underwater speaker (Model UW30, Lubell, Columbus, Ohio, USA) was used to project the acoustic stimuli of the different boats inside the experimental tank. A playlist with the 8 selected wave files was created. The playlist was projected using the "loop mode" and random function of the Avisoft-SASLab recorder software (Avisoft Bioacoustics) through the stereo output of a PC connected to a power amplifier (type 2713, Bruel & Kjer - Naerum, Denmark) (see Figure 1).

The acoustic stimuli projected inside the tank were recorded using the same acoustic equipment and set-up employed for the acquisition phase. The hydrophone was placed at a 0.5 m depth in the centre of the tank

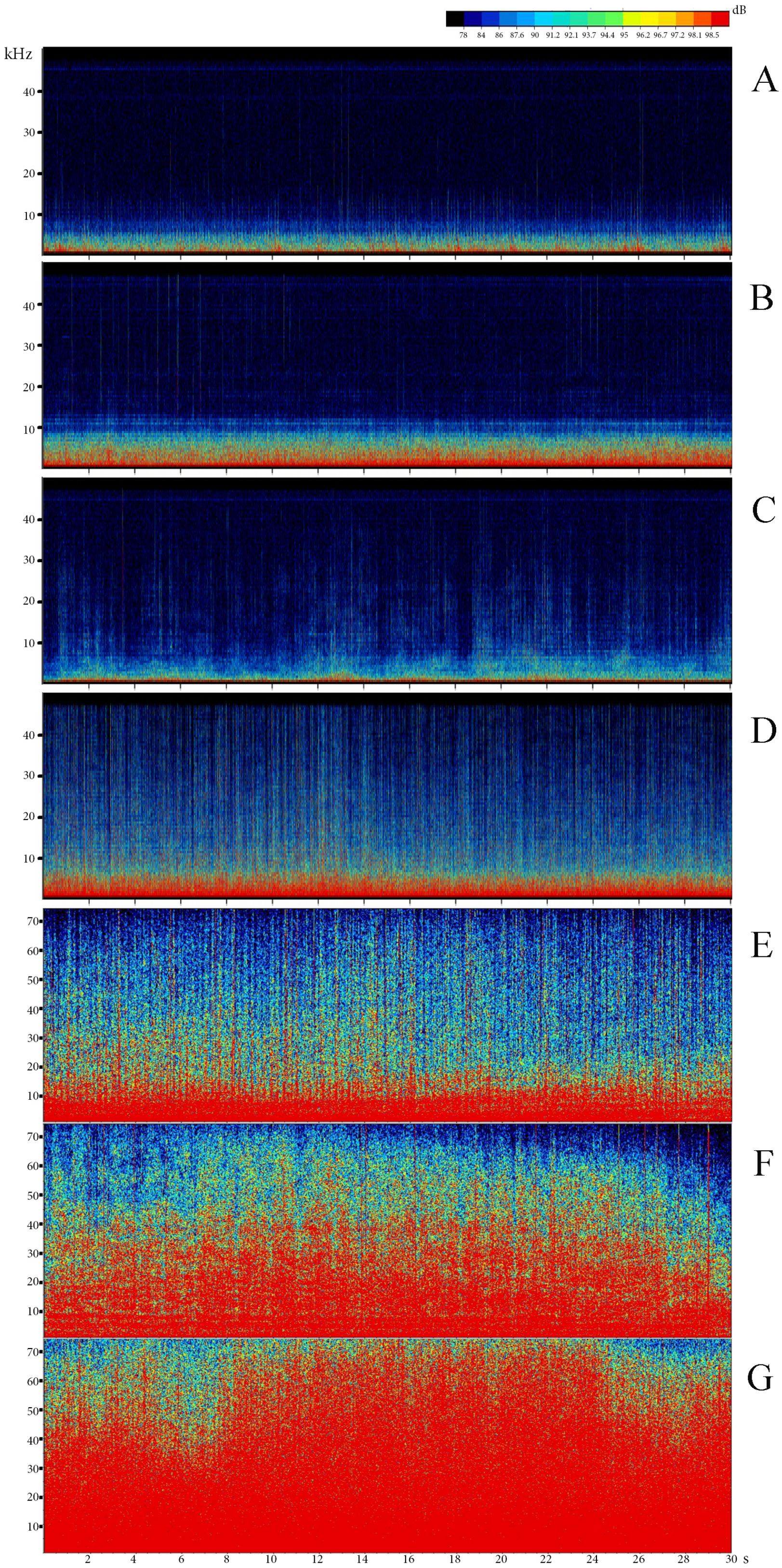

Figure 2. Spectrogram of the experimental tank background noise and of different boat noise stimuli: frequency (kHz) versus time (s). A) Experimental tank environment; B) Recreational boat 1; C) Recreational boat 2; D) Recreational boat 3; E) Recreational boat 4; F) Recreational boat 5;

G) Hydrofoil; H) Fishing boat; I) Ferry boat. The intensity is reflected by the colour scale (dB re 1 μPa, root mean square, 1024-sample FlatDown window, sampling frequency 96 kHz).

4. VIDEO MONITORING SET-UP

The video system was used to monitor the behaviours of prawns and was synchronized with the system used to record the acoustic signals. Videos for behavioural monitoring were recorded with a camera with housing (model Hero 2, GoPro Inc., USA) placed on top of the experimental tank. The camera was linked to a Personal Computer and the files were managed by Adobe Premiere Pro CC (Adobe Systems Inc., USA).

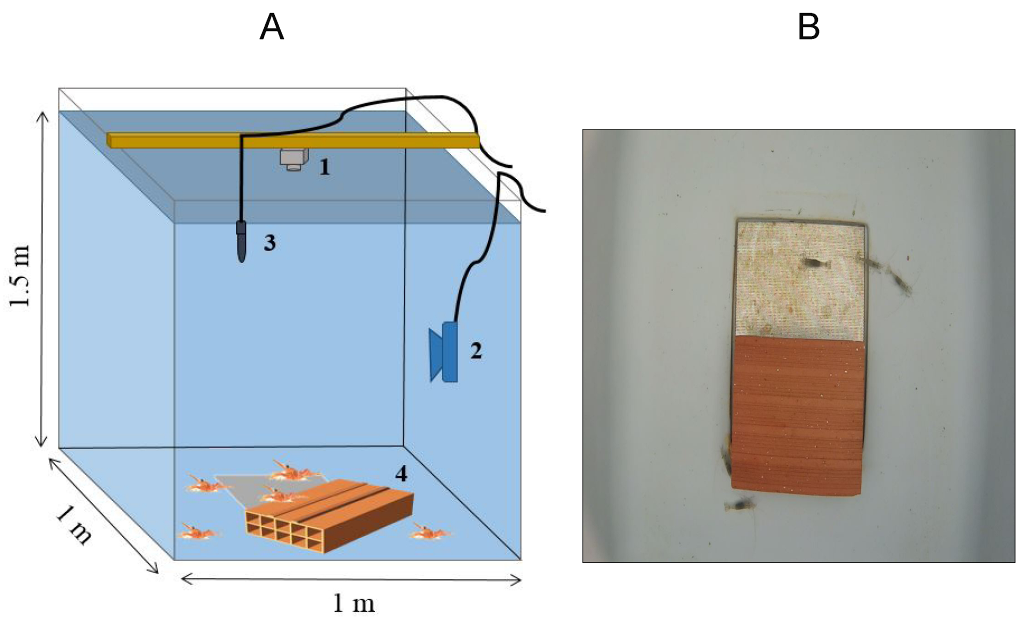

Figure 3. Experimental set up of the tank-based experiments adopted during all the tests. A) Scheme of the tank equipment: 1. GoPro camera, 2. underwater loudspeaker, 3. hydrophone and 4. calcarenitic shelter; B) Real image from the video recordings.

In order to detect the behavioural states and events of the prawns, the videos were analyzed using a visual continuous sampling procedure (each observed event from each animal was annotated in a sampling table). Startle responses, encounters between subjects, tail flipping events, time spent in and outside the shelter, and time spent walking and resting, as described in Table 1, were analyzed. A focal animal sampling technique modified from Altmann, 1974 was used to analyze the images in slow motion. For each 10 minutes of sampling, a value as the sum of the events for each specimen was recorded.

Table 1 Description of behavioural actions by common prawn (*Palemon serratus*) based on videotapes visualizations.

Behaviours	Description
Startle response	Abrupt response to a sudden stimulus that is unexpected and alarming. The movement may translate the whole body or move only limited parts (Bullok, 1984)
Encounters	Two or more individuals walk towards each other until they encounter each other or make contact with some part of the body without a thread display. Two or more individuals walk towards each other until they encounter each other or make contact with some part of the body without a thread display
Outside shelter	Time spent by the prawn outside the shelter
Inside shelter	Time spent by the prawn inside the shelter
Walking	The prawn uses its legs to move itself to another location
Resting	The prawn maintains its position
Tail flip	Rapid flexion of the extended abdomen, one or more times, which results in propelling the prawn to a new location (Buscaino et al., 2011; Lavalli and Herrnkind, 2009)

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