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Behavioural and biochemical stress responses of *Palinurus elephas* after exposure to boat noise pollution in tank

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ABSTRACT

This study examined the effects of boat noise on the behavioural and biochemical parameters of the Mediterranean spiny lobster (*Palinurus elephas*).

The experiment was conducted in a tank equipped with a video and audio recording system. 18 experimental trials, assigned to boat noise and control conditions, were performed using lobsters in single and group of 4 specimens. After a 1 h habituation period, we audio- and video-recorded the lobsters for 1 h. During the experimental phase, the animals assigned to the boat groups were exposed to boat noise pollution (a random sequence of boat noises). Exposure to the noise produced significant variations in locomotor behaviours and haemolymphatic parameters. Our results indicate that the lobsters exposed to boat noises increased significantly their locomotor activities and haemolymphatic bioindicator of stressful conditions such as glucose, total proteins, Hsp70 expression and THC when tested both singly and in groups.

1. Introduction

Over the past 50 years, anthropogenic activities, in particular shipping traffic, have produced increasing background sea noise pollution (Ross, 2005; Hildebrand, 2009). Human activity can generate alterations and other significant changes in marine habitats and animals (Myrberg, 1990; McIntyre, 1995; Popper et al., 2004). In recent years, many studies have evaluated the effects of anthropogenic acoustic disturbance on marine organisms (Santulli et al., 1999; Scholik and Yan, 2001; Sarà et al., 2007). These sounds, associated with shipping, seismic surveys, sonar, and many other anthropogenic sources, induce several types of effects in fish and marine mammals (Au et al., 1974; Myrberg, 1990; Thomas et al., 1990; Engås et al., 1996; Scholik and Yan, 2001; Sun et al., 2001; Amoser and Ladich, 2003; NRC, 2003; Romano et al., 2004; Smith et al., 2004; NCR, 2005; Popper et al., 2005; Sandström et al., 2005; Wysocki et al., 2006; Codarin et al., 2009; Buscaino et al., 2010; Filiciotto et al., 2013). However, few studies have evaluated the effects of acoustic stimuli on aquatic crustaceans (Christian, 2003; Andriguetto-Filho et al., 2005; Celi et al., 2013; Wale et al., 2013).

In aquatic crustaceans, the impact of environmental stress can directly affect haematological parameters (Perazzolo et al., 2002; Celi et al., 2013). THCs and DHCs have been used to assess crustacean health and the effects of stressful conditions. Decreases in the THC under stressful conditions have been reported for several marine crustacean species (Le Moullac et al., 1998; Sánchez et al., 2001). Moreover, although the response of the DHC to different stressors is not well understood; it has been used as a stress indicator in crustaceans (Jussila et al., 1997; Johansson et al., 2000). Changes in the levels of other plasma components have been described in shrimp under several conditions. Hyperglycaemia and total proteins are typical stress responses to harmful physical and chemical environmental changes, including hypoxia and exposure to air during commercial transport (Le Moullac et al., 1998; Durand et al., 2000; Speed et al., 2001). Hyperglycaemia has been associated with increased circulating crustacean hyperglycaemic hormone (CHH) titres (Stentiford et al., 2001; Lorenzon et al., 2004) and has been used as an index to assess CHH activity and environmental stress. Blood protein levels fluctuate with changes in environmental and physiological conditions and play fundamental

Abbreviations: BOAT, Boat noise condition; CTRL, Control condition; BSA, bovine serum albumin; CHH, crustacean hyperglycaemic hormone; DHC, differential haemocyte count; Hsp, heat shock protein; PBS, phosphate buffered saline; PC, protein concentration; SIM, Social Interaction Module; TBS, Tris-buffered saline; THC, total haemocyte count.

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roles in the physiology of crustaceans, from O_2 transport to reproduction to stress responses (Chang, 2005; Lorenzon et al., 2011). These findings suggest that total protein concentration levels (PC) can potentially function as a stress indicator that can be used to monitor the health status of crustaceans (Lorenzon et al., 2011). The bioindicators of stressful conditions in crustaceans include Hsp70 expression (Snyder and Mulder, 2001; Liberge and Barthélémy, 2007). Little is known about the effects of noise on Hsp expression. Wu et al. (2001) showed that Hsp70 expression increased after exposure to a stressful noise in humans, and Hoekstra et al. (1998) reported that the expression of Hsp70 (but not Hsp30, Hsp60 or Hsp90) is increased in birds after exposure to a loud noise.

Behavioural observations, in combination with physiological assessment, may provide a more complete understanding of the homeostatic perturbations of an organism due to external or internal stress stimulus. For example, quantifiable behavioural changes in an organism that are associated with stress and toxicant exposure provide novel information that cannot be gained from traditional toxicological methods, including short-term and sub-lethal exposure effects and the potential for mortality (Bridges, 1997; Henry and Gary, 1986; Saglio and Trijasse, 1998).

A combination of behavioural and physiological approaches may have significant relevance in crustaceans, for which the behavioural patterns in response to stress conditions are not yet well known.

For example, Payne et al., 2007 found that lobsters exposed to very high as well as low sound levels experienced no effect on delayed mortality or damage to the mechanosensory system associated with animal equilibrium and posture. Celi et al. (2013) observed that red swamp crayfish showed altered aggressive behavioural patterns and changes in the components of the haemato-immunological system, such as serum glucose concentration; protein concentration; agglutinating activity; and THC, DHC and Hsp70 expression, when exposed to an acoustic stimulus in a frequency band of 0.1–25 kHz, clearly reflecting a stress condition.

The European spiny lobster *Palinurus elephas* is a common crustacean species along the Mediterranean and northeastern Atlantic coasts (Hunter 1999). This species, which is primarily active at night for feeding and reproduction (Goni and Latrouite, 2005), represents one of the major targets of Mediterranean artisanal fisheries, and while catches are now reduced and sporadic, this fishery has a long history (Goni and Latrouite, 2005; Groeneveld et al., 2006).

In mobile species such as the European spiny lobster, locomotor movement is an important link between the behaviour of individuals and ecological processes (Herrnkind, 1983; Spanier et al., 1988; Lawton and Lavalli, 1995). Density-dependent mechanisms underlying the structure and dynamics of populations and communities are often sensitive to short-term changes in the spatial distribution of individuals due to movement (Milinski and Parker, 1991).

Moreover, spiny lobsters, like other arthropods, produce acoustic signals (Patek, 2002; Patek and Oakley, 2003; Patek and Baio, 2007; Bouwma and Herrnkind 2009; Buscaino et al., 2011a, 2011b). Some authors have assumed that lobsters only produce sounds in an antipredatory context (Patek 2001, 2002; Patek and Oakley, 2003; Bouwma and Herrnkind, 2009), while others suggest that sounds may be used in social context as well (Mercer, 1973).

Although the ability of lobsters to perceive acoustic signals has not yet been documented and even the sensitivity of lobsters to different frequencies remains unknown (Buscaino et al., 2011b), the hypothesis that *P. elephas* is able to perceive boat noise appears reasonable because of the wide bandwidth of acoustic signals generated by boats (from a few Hz to more than 20 kHz; Codarin et al., 2009; Hildebrand, 2009), as Celi et al. (2013) have already observed in crayfish exposed to a linear sweep with a frequency range of 0.1–25 kHz.

In consideration of the concerns described above, the present study investigated the behaviour (locomotor states and acoustic emissions) and biochemical (haemolymphatic parameters) responses of the European spiny lobster (*P. elephas*) after the exposure to acoustic pollution consisting of boats noises. The locomotor analysis automatically estimated the movement/position events of lobsters, offering a novel, high-throughput method of measuring the relationship of the lobster compared to traditional manual analyses. The biochemical effect on the lobsters was evaluated by estimating the serum glucose concentration; total protein concentration; and THC, DHC and Hsp70 expression as stress indexes.

2. Materials and methods

2.1. Animal housing and experimental design

The present study was carried out at the Institute for the Marine and Coastal Environment of the National Research Council (CNR-IAMC) of Capo Granitola (SW Sicily, Italy). Approximately 80 specimens of European spiny lobster (*P. elephas*) were captured in May 2013 at a depth of 20–25 m near Mazara del Vallo (SW Sicily, Italy) by fishermen using a commercial trammel net (54-mm inner-panel mesh size, 1200-m length). After capture, the lobsters were transferred to two indoor circular PVC tanks (2.35 m diameter and 1.5 m depth) for a month-long acclimation period. Before the beginning of the acclimation phase, males were marked using a non-toxic water resistant paint (Markservice, Milan, Italy) that was spread on the central portion of the lobsters' carapace to distinguish them from the females.

The lobsters were fed with frozen molluscs, shrimps and fish ad libitum. After the acclimation period, 36 lobsters (18 males and 18 females) of 301.96 ± 69.8 g in weight and 7.73 ± 0.69 cm in carapace length (mean ± SD), individually or in groups of 4 individuals, were randomly collected from the holding tanks, were assigned to the trials and were used in only one experiment to meet the assumption of experimental independence. For the experimental procedure, we used only lobsters that had not recently molted. The lobsters were released into the centre of an experimental tank that was identical in size, shape and water composition with the holding tanks. No shelter was present in the experimental tank. The lobsters were deprived of food for 5 days before the start of the experimental trials. All animals were kept under natural photoperiods.

The holding and experimental tanks were equipped with an independent flow-through seawater system from a common source (25 ± 3.7 l min^{-1}; mean ± SD). Salinity was 36.4 ± 0.81 ppt (mean ± SD), and the temperature was 18.61 ± 0.39 °C (mean ± SD) during the entire study period.

The experiment was performed during the month of June 2013. During the experimental procedure, the lobsters were exposed to two different acoustic conditions (Fig. 1):

- Boat noise condition (BOAT) – an underwater loudspeaker reproduced the noise from a marine area with high anthropogenic acoustic pollution using a random sequence of boat noises, including recreational boats, hydrofoils, fishing boats and ferry boats;
- Control condition (CTRL) – lobsters were exposed only to the low-level noise of the experimental tank's background noise. The loudspeaker, even if turned off, was not removed from the tank during the control trials to maintain the same landscape as the treatment trials.