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Use of multiple cell and tissue-level biomarkers in mussels collected along two gas fields in the northern Adriatic Sea as a tool for long term environmental monitoring

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ABSTRACT

As a consequence of the growing global demand of energy supplies, intense oil and gas exploration and exploitation programs have been carried out worldwide especially within the marine environments. The release of oil-derived compounds in the sea from anthropogenic sources both as effluents or accidental spill is perceived as a major environmental concern. An approach based on a combination of biomarkers and the distribution of some classes of environmentally relevant pollutants was used to investigate the occurrence of a stress syndrome in mussels (*Mytilus galloprovincialis*) collected at three gas platforms placed in two distinct oceanographic districts within the Adriatic Sea. Biological responses were integrated by a ranking algorithm which demonstrated both a range of biological effects reflecting exposure gradients and a temporal related trend in the investigated responses. The overall results demonstrate a moderate to absent pollution from studied gas platforms with low but remarkable biological disturbance in sentinel organisms.

1. Introduction

Global energy demand has grown rapidly since the mid-1970s. This is predicated to continue being an adequate supply of energy essential to industrial growth. Increasing energy consumption is required to preserve human welfare, and support future industrial growth in both developed and developing countries. The increase of energy supplies have became a priority interest for governors and rulers and since the most of the world's energy consumption derives from the combustion of fossil fuels, large-sized exploration and exploitation programmes have been carried out worldwide by the main oil companies (US-EIA, 2011). Nowadays, more than 110 gas platforms are active in the Adriatic Sea, a semi-enclosed basin characterized by a low water recirculation rate and an elevate anthropic pressure associated with unsustainable fishing and the high inputs of environmental contaminants. Due to the increasing amount of produced waters (PWs), a by-product of oil extraction processes released into the marine environment, offshore oil and gas drilling activities have become source of ecological concern by the scientific community (Brooks et al., 2011; Gomiero et al., 2011; Gorbi et al., 2008). Even though considering a quick dilution in surrounding seawater, marine organisms could be exposed to low but continuous concentrations of this complex effluent enriched of oil derived hydrocarbons, non volatile dissolved organic material and elevated concentrations of trace metals. Differently from major oil spills which can occur occasionally and receive considerable public attention producing acute and easily detectable effects, oil-derived compounds which enter the sea as small amounts are released over long periods, thus creating chronic exposure of organisms to oil and its component chemical species. In these cases, effects are very often difficult to detect as the concentrations of chemicals are under detectable limits and there may be a strong gradient from a high to a low oil concentration as a function of distance from the source. Under these conditions the simply chemical based approach can fail to report the effective loss of water quality near off-shore oil and gas rigs.

The aim of this work was to apply a multidisciplinary approach based on a selection of sensitive sublethal stress indexes (biomarkers) at different levels of biological organization (molecular/

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cellular/tissual/organismic) to assess the impact of three gas platforms located into two distinct oceanographic districts in the Adriatic Sea (Fig. 1). Furthermore, biological responses were related to the bioaccumulation trend of environmentally relevant organic and inorganic pollutants. Native mussels were collected both close to (CA) and far from (FA) the platforms' galvanic anode and at a control site (C). Biological responses were than integrated thorough a novel algorithm the "Mussel Expert System" (MES) aiming at drawing up indices to rank the different stages of pollutant-induced stress syndrome since environmental managers need to be able to translate biomarker data into an actual health risk index (Dagnino et al., 2007). Hence, to obtain a highly informative and reliable collection of biological responses, the battery of biomarker was designed fulfilling some of the requirements suggested by UNEP, ICES and OSPAR guidelines (Davies and Vethaak, 2012; UNEP, 1999). A multiple and sequential tiered approach was developed to set up a cost-effective tool which Environmental Agencies could easily afford to use in routine and large scale biomonitoring programmes (Viarengo et al., 2007). A first tier, referred to as a screening phase, involved the use of a highly sensitive, low-cost biomarkers such as lysosomal membrane stability and mussel survival on air rates which enabled the detection of high levels of pollution induced stress. A second tier, when an alteration in lysosomal stability was established, involved an extended suite of biomarkers to fully characterize the level of the stress syndrome as well as the toxicity mechanisms in the investigated sentinel organisms. Thus, lysosome membrane stability (LMS), catalase activity (CAT), malondialdehyde concentrations (MDA), lysosomal lipofuscin (LF) and neutral lipids (NLi) content, which are sensitive to stress at a molecular and cellular level, were associated to lysosome/cytoplasm (Lys–Cit) ratio, a lysosomal structural changes test which can be straightforward and very useful in cases of extreme symptoms of pollutant's damage. Stress on stress (SoS) and Condition Index (CI) were measured to detect stress at the organism level, giving indication of the potential survival capacity of the animals as well as their general energy balance. These markers, in particular, are essential to relate the effects of pollutants on individuals to possible changes at population level. Furthermore, in order to investigate both the occurrence and the effects of as many groups of toxic compounds as possible, some biomarkers of exposure, like alkaline labile phosphate (ALP) and metallothionein content (MT), were used to assess the exposure to endocrine disrupting compounds and heavy metals, respectively (Blaise et al., 1999; Viarengo et al., 1999). Finally, the appearance of genotoxic alterations in sentinel organism was evaluated by the frequency of micronuclei (MN) which provides information on less reversible effects resulting from chromosome breakage during cell division. Indeed, it can be considered a good indicator of genetic damage accumulated during the cell life span providing an indication of what is the extent of the genotoxic effects of pollutants (Viarengo et al., 2007). The wide array of biological responses measured at different levels of the biological organization is expected to report the earliest warning signals of environmental disturbance related to many of those environmental stressors connected with off-shore gas exploitation and production activities. The overall results of this work are expected to provide an ecotoxicological based support for long term monitoring of offshore exploitation and production activities as well as to support the development of environmental risk assessment in marine environments.

2. Material and methods

2.1. Chemicals

All chemicals were of analytical grade and purchased from Sigma–Aldrich, if not otherwise stated.

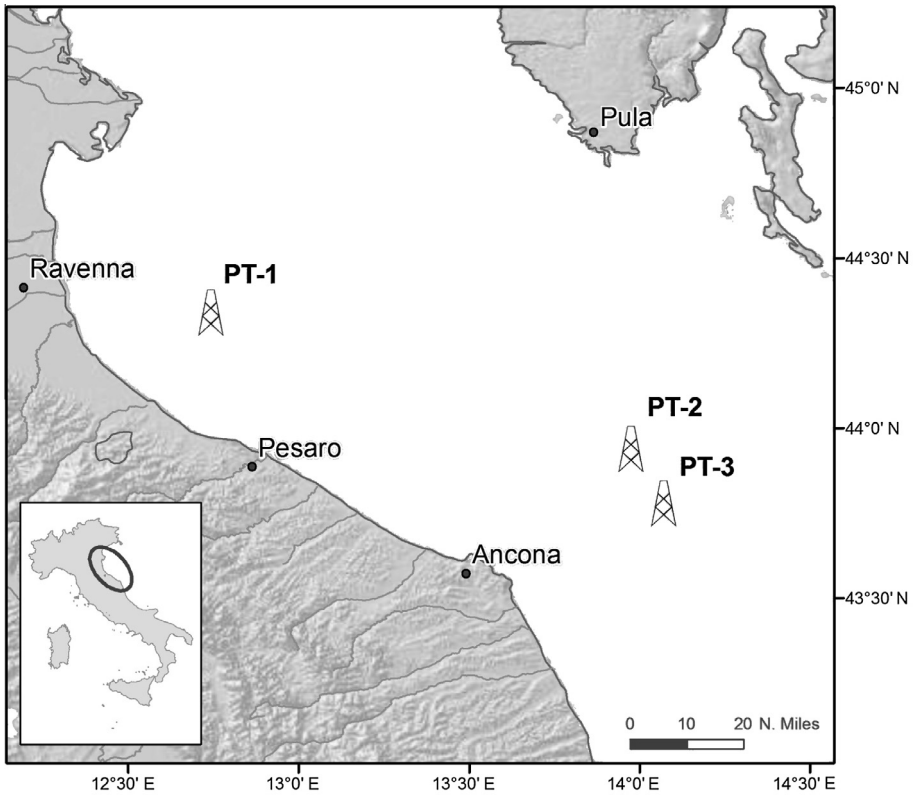

Fig. 1. Adriatic Sea, study area with the location of the monitored gas platforms.

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