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Seasonal antioxidant responses in the sea urchin *Paracentrotus lividus* (Lamarck 1816) used as a bioindicator of the environmental contamination in the South-East Mediterranean

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ABSTRACT

In this study, sea urchin *Paracentrotus lividus* were sampled seasonally at three stations during 2012 in the coastal areas of the Gulf of Annaba (southeast Mediterranean). For all sea urchins, the gonad index was calculated to determine sea urchin reproductive status. Moreover, a set of biochemical parameters, including biomarkers and oxidative stress parameters, was measured in gonads. The pesticides and physiochemical parameters were measured and dosed in sea water.

The results obtained highlighted that the levels of pesticide were generally low and below those commonly applied by environmental quality standards (EQS), indicating that no alarm state is currently present in the Gulf of Annaba. In addition to pollution, seasonal change is an important factor influencing biomarker activity, and the significant increases in biomarker levels in spring are a major observed trend. This activity may also be related to reproductive status. Seasonal variability was confirmed by the significant results of the Kruskal-Wallis test and by the high degree of divergence between seasons in PCA, with a total of 83.83% of variance explained.

These results indicate that environmental factors that vary seasonally may affect the antioxidant status of the sea urchin *Paracentrotus lividus*.

1. Introduction

Pollution of the marine environment is a global concern due to the devastating effects of contaminants, which are reaching increasingly alarming levels. According to the European Marine Strategy Framework Directive (2008/56/EC), a marine habitat with good environmental status has contaminant concentrations that do not give rise to pollutant effects (European Commission, 2008a). The presence of pesticides in different coastal and marine habitats around the world has already been detected on the coast of Hong Kong in China (Xu et al., 2015b), the Antarctic coast of New Zealand (Emnet et al., 2015), the bay of Vilaine in France (Caquet et al., 2013), the Caribbean and Pacific coasts of the United States (Menzies et al., 2013), the northern Adriatic sea of Italy (Loos et al., 2013), the Baltic sea of Germany (Nödler et al., 2013), the Mar Menor lagoon in Spain (Moreno-González et al., 2013), the Thermaikos Gulf in the Northern Aegean sea of Greece (Arditsoglou and

Voutsas, 2012), the Mi-Black sea Coast in Turkey (Binnur Kurt and Boke Ozkoc, 2004) and even at polar latitudes (Chernyak et al., 1996; Hoekstra et al., 2002; Sobek and Gustafsson, 2004). The presence of pesticides can pose a serious problem and their accumulation, bioamplification and transformation in aquatic ecosystems is a real risk for human health, fauna and the environment (Navarro et al., 2000). The works of Navarro et al. (2004); Maund et al. (1997); Sundaram (1997); Hadfield et al. (1993); Ramesh et al. (1990); Mhlanga and Madziva (1990); Rico et al. (1989); Najdek and Bazulic (1988); Rivera et al. (1986) have revealed ecological damage caused in distinct aquatic ecosystems following the generalized use of agricultural pesticides. The exposure of aquatic organisms to pesticides can lead to the production of reactive oxygen species (ROS) (Üner et al., 2006; Ahmad et al., 2000), causing an imbalance between the production of ROS and endogenous antioxidant activity (Kamat et al., 2000). The physiological response of marine organisms is strongly dependent on fluctuations of

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biotic and abiotic factors such as salinity, oxygen concentration, and temperature and food availability, resulting in difficulties in interpreting the biological effects exerted by xenobiotics (Camus et al., 2004; Manduzio et al., 2004). Changes in biomarker levels may simply be a natural part of the annual physiological cycle of the species and quite unrelated to changes in exposure to chemical pollution (Sheehan and Power, 1999).

The Gulf of Annaba is constantly threatened due to its proximity to port activities, runoff, urban and industrial discharge and the most active agricultural regions of eastern Algeria. The benthic fauna and flora living in close contact are appropriate representative samples of great importance for explaining the link between the health of aquatic organisms and contamination levels. Until now, the pollutants detected most often have been heavy metals and nutrient salts. The main objective of this study was to investigate whether seasonal variation may influence the biochemical parameters of the sea urchin. An integrated biological approach was chosen that implements a combination of physical, chemical and biological measures in the framework of a relatively comprehensive study using the marine invertebrate belonging to the phylum *Echinodermata*. The sea urchin *Paracentrotus lividus* is an edible echinoid found in great abundance on the Mediterranean coast (Tejada et al., 2013). Its gonads make it a highly appreciated sea food in various countries including France, Spain, Italy (Fernández-Boán et al., 2013) and Japan (Powell et al., 2014). Due to its value as a luxury food (Cook and Kelly, 2007), it has also shown itself to be an excellent bioindicator of pollution in the marine environment due to its sedentary habits and known sensitivity to pollutants (Soualili et al., 2008). It has been used in several studies as a bioindicator of local pollution (Angioni et al., 2014; Bayed et al., 2005; Demnati et al., 2002; Coteur et al., 2003). In recent years it has acquired importance as a model organism in marine ecosystem biomonitoring programs (Fabbrocini et al., 2010; Bellas and Paredes, 2001).

2. Materials and methods

2.1. Sampling station

The study was carried out over four seasons in 2012: winter

(February), spring (April), summer (July) and autumn (October). Three sampling stations were selected in the Gulf of Annaba (Algeria) to implement an integrated monitoring strategy. Fig. 1. The Gulf is a large bay located on the eastern Algerian coast, 600 km from Algiers and 100 km from the Tunisian border, open to the Mediterranean Sea to the north, and extending 40 km between Cap Rosa in the east and Cap Garde in the west (Belabed et al., 2013). The characteristics of the stations selected are shown in Table 1.

2.2. Environmental characterization

During sampling, temperature, pH, salinity, turbidity and dissolved oxygen were measured in situ with field multi-parameters. Water samples were collected in plastic containers that were sealed and transferred to the laboratory in ice packs to determine the following chemical parameters: nitrate (NO_3^-), nitrite (NO_2^-), ammonia nitrogen (NH_4^+) and orthophosphate (PO_4^{3-}) using the manual colorimetric methods of Aminot and Kérouel (2004b). For pesticide determination, freshwater samples were collected in amber glass bottles and kept at low temperature in the laboratory, where the water was filtered and stored. Five pesticides were analyzed with ELISA kits (Atrazine, Diuron, and glyphosate from Abraxis; Nonylphenol from Tokiwa Chemical Industries). Each kit consisted of eight separate 12-well immunoreader strips precoated with specific antibodies. The assay was performed following the manufacturer's guidelines and the readings were performed using an MR – 96 A automatic Elisa plate reader from Mindray.

2.3. Animal sampling and sample preparation

Specimens of *Paracentrotus lividus* 50 mm in diameter (market size) were collected from three stations in the Gulf of Annaba. The samples were transported in a cooler with oxygenated seawater. The specimens from each station were measured and dissected in the laboratory. The gonads were weighed, immediately removed and homogenized in 20 mM Tris buffer (pH 7.6) containing 1 mM EDTA, 0.5 M sucrose, 0.15 M KCl and 1 mM DTT. The homogenate was centrifuged at 9000g for 20 min at 4 °C. The supernatant was stored at – 40 °C until analysis.

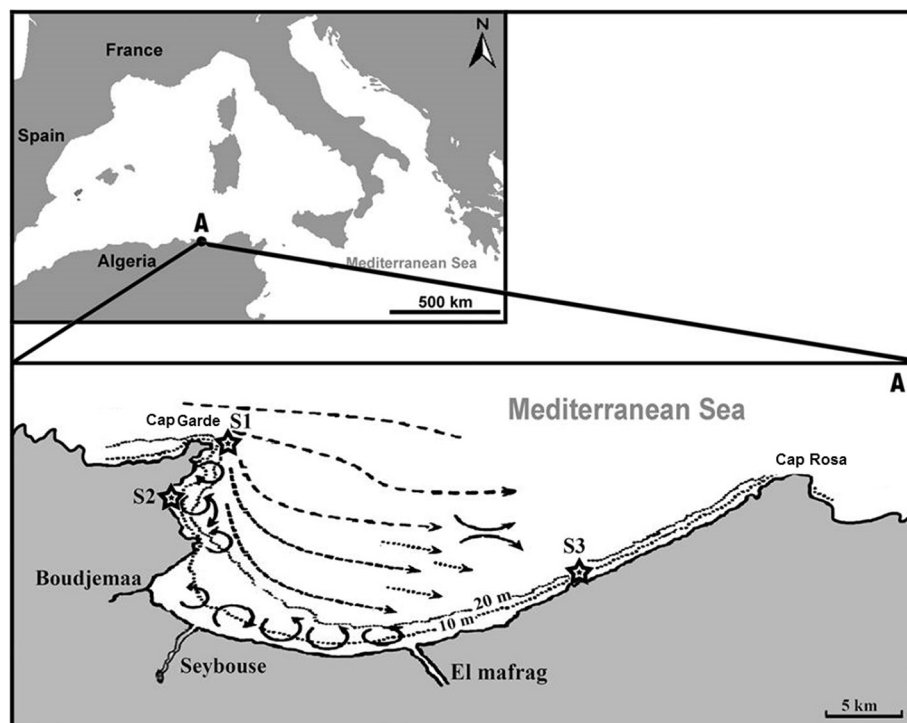


Fig. 1. Map of the three sampling stations (S.1, S.2 and S.3) and of the main processes governing the marine dynamic inside the Annaba Gulf (Algeria).

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