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Limpet (*Patella sp*) as a biomonitor for organic pollutants. A proxy for mussel?

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ABSTRACT

The scarcity of the most widely used species for assessing marine pollution (mussels) in some areas brings out the need to test the use of a different organism. In this study, 11 sampling sites along the Atlantic Spanish coast were selected and both mussels (*Mytilus galloprovincialis*) and limpets (*Patella sp.*) were analysed for PAHs, PBDEs and PCBs. The concentrations of the different pollutants in both species followed the same general distribution allowing us to differentiate polluted and unpolluted sites using any of them. Although the concentrations found in limpets were generally lower than those measured in mussels, a good correlation was observed for most of the groups of pollutants and also for every individual congener. A conversion factor was proposed for most of the individual PAH and PCB congeners, allowing the conversion of limpet concentration into mussel concentration that can be directly applied in assessments using environmental criteria derived for mussels.

1. Introduction

The most widely used species for marine pollution studies is mussel, *Mytilus spp.*, as it is a filter feeder, sessile, easy to get and present in most worldwide coasts. However, sometimes it is necessary to have an alternative species for areas where mussels are scarce or even absent.

Other bivalves, mainly oysters (*Crassotrea spp*), have been widely used as substitutes and/or complement for mussels in pollution control programs (Beliaeff et al., 1998; Cantillo, 1998; Oros et al., 2005; Sole et al., 2000). Other possible useful substitutes of mussels, as bioindicator, are limpets (*Patella sp*). Limpets are the dominant grazers on exposed shores of north-west Europe (Jenkins et al., 2001) and as such, the study of pollutants accumulation in this species can also be relevant as a potential transfer of contaminants through the food web by predation of contaminated limpets.

The idea of using limpets as an alternative group for mussels is based on the abundance of limpets in those areas of the rocky shores where mussels are scarce or absent, and the need to cover the entire coastline in pollution monitoring programs. The use of limpets in biomonitoring programs has been rather limited to date (Reguera et al., 2018) in the case of Polycyclic Aromatic Hydrocarbons –PAHs- and Organochlorine Compounds (Bartolomé et al., 2011; Corbella Tena and Garcia Montelongo, 1999; Gianguzza and Orecchio, 2006; Peña-Méndez et al., 1999; Rodríguez Delgado et al., 1999) and normally limited to a small sampling area and/or after an oil spill. There are no previous references of measuring PBDEs in *Patella*. A bit more common are the works measuring metal pollution in limpets (Bergasa et al., 2007; Collado et al., 2006; Cravo and Bebianno, 2005; Duarte et al., 2012; Hamed and Emara, 2006; Ramelow, 1985).

Both groups (mussels and limpets) are intertidal and microphagous animals which can ingest pollutants with food particles and accumulate them in their tissues. But, on the other hand, they have different feeding strategies: while limpets scrape particles and microalgae from the rock with their horny rasping tongues, mussels collect food particles from the water by filter feeding (Naes et al., 1998).

The implementation of these pollution control programs in Europe is now determined by the Marine Strategy Framework Directive (EC, 2008), which demands an approach focused not only on the analytical chemistry of pollutants, but also on evaluating their effects on the ecosystems. And it has to be applied throughout the coast, even in those areas where typical monitoring species are not available.

Polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) are normally a fixed core in the main lists of organic pollutants measured worldwide (European_Commission, 2008; Kimbrough et al., 2008; OSPAR, 2010) and cover a wide range of pollution sources including industrial, urban and traffic.

No previous works on measuring different organic pollutants, namely PAHs, PCBs and PBDEs both in mussels and limpets have been found in the bibliography.

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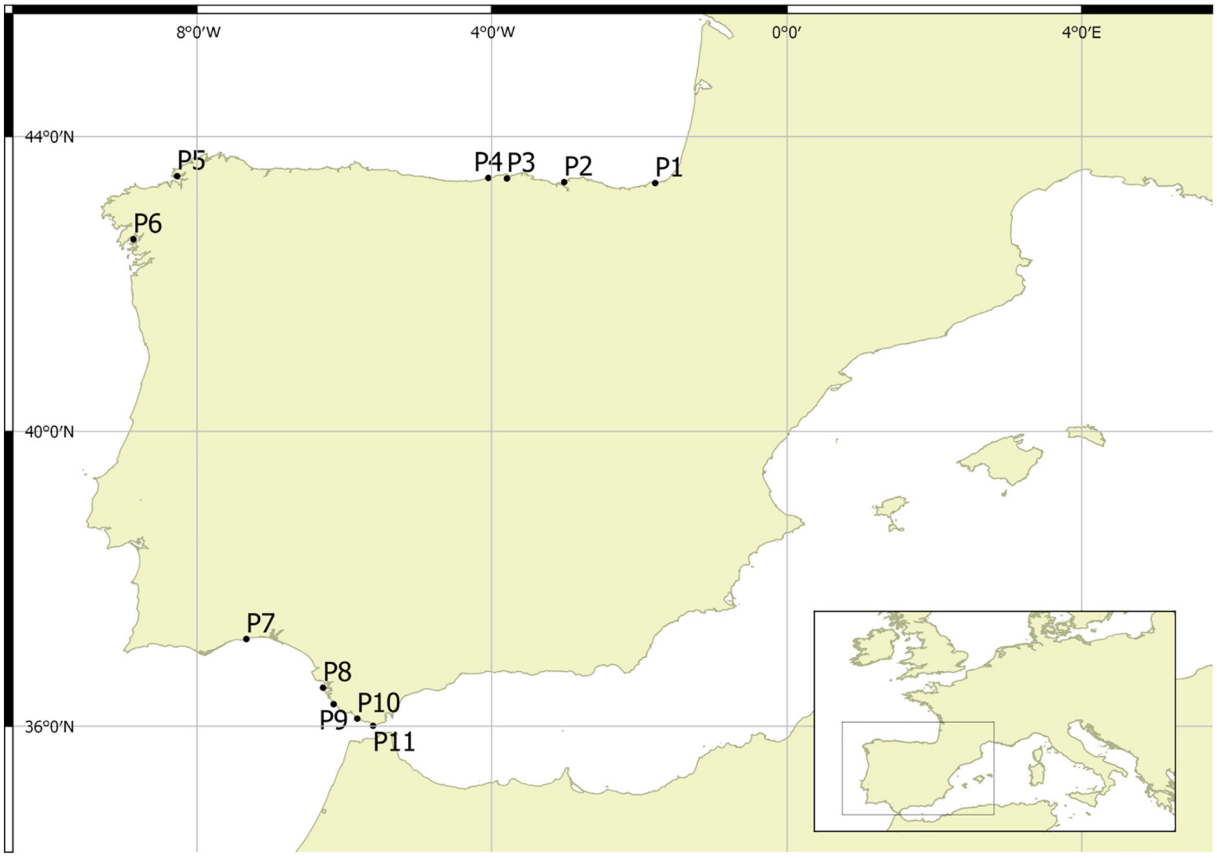

Fig. 1. Limpet and mussel sampling locations.

The aim of this study was to 1) evaluate the concentration of different organic pollutants in both mussels and limpets along a wide coastal area in Spain and 2) assess the suitability of limpets for monitoring purposes. A secondary objective was also trying to derive a conversion factor to directly compare concentrations in limpets to those environmental criteria derived for mussels, allowing the use of limpets as a substitute or proxy for mussels where they cannot be used.

2. Materials and methods

2.1. Location

Mussels and limpets were sampled in November 2014 in 6 sampling sites along the Northwest coast of Spain, P1 to P6 (all these sites except P2 and P3 had been also sampled in 2013) and in 5 sampling sites along the Southwest coast of Spain, Gulf of Cadiz, P7 to P11.

The situation of the stations can be checked in Fig. 1 and a list of the samples with the coordinates in Table S1.

These sites were selected taking into account that both species should be present and abundant enough to sample both more than one year. Besides, the sampling locations represent different grades of pollution as described previously (Soriano et al., 2006, 2007).

2.2. Sample handling

Mussels and limpets were sampled simultaneously in the same sampling points in November 2013 and 2014.

Samples were collected by hand at low tide and stored at −20 °C until analysis. At least 50 specimens of each species were sampled in each location and sampling period. Once the samples were defrosted, the soft tissue was removed and samples were homogenized.

Mussels in the size range of 35–60 mm and limpets in the size range of 25–55 mm were selected for analysis.

In the case of mussels, three subsamples for each sample site and year were prepared at the lab and the subsamples were then individually analysed. The values presented in this study are the mean values of the three subsamples. For limpets only one sample was prepared for each sample site and duplicate analysis were done randomly to assure repeatability.

Due to the scarcity of the limpet sample from Cadiz, it was not analysed for PAH content and so the mussel results for this sample site and group of pollutants are not included.

2.3. Chemical analyses

2.3.1. Polycyclic aromatic hydrocarbons

About 10–15 g of wet homogenate tissue from each species, station and sampling period were Soxhlet extracted with a 1:3 acetone:hexane mixture for 8 h and analysed by High Performance Liquid Chromatography (HPLC) as described elsewhere (Soriano et al., 2006; Viñas et al., 2009). In summary, samples to be analysed by HPLC were submitted to a clean-up step by column chromatography on deactivated alumina (10% water) and hexane elution.

The 12 PAHs (phenanthrene - Phen-, anthracene - Ant-, fluoranthene –Fla-, pyrene –Pyr-, chrysene –Chrys-, benz[*a*]anthracene –BaA-, benzo[*b*]fluoranthene –BbF-, benzo[*k*]fluoranthene –BkF-, benzo[*a*]pyrene -BaP, benzo[*ghi*]perylene –BghiP-, dibenz[*ah*]anthracene -dBahA- and indeno[1,2,3-*cd*]pyrene –IP-) were determined by HPLC (HP 1100, Agilent Technologies) coupled with a wavelength programmable fluorescence detector (HP 1036, Agilent Technologies). The column (Zorbax Eclipse PAH, Agilent Technologies) was kept at 23.5 ± 0.1 °C and eluted with a methanol:water gradient.

Certified solutions, supplied by Dr. Ehrenstorfer were used for quantification, using a multilevel calibration at six points between 10