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Effects of organic pollution on biological communities of marine biofilm on hard substrata

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ABSTRACT

We examined the effect of organic enrichment on diatom and bacterial assemblages of marine epilithic biofilms on two locations in the Mediterranean, one situated in Spain and the other in Greece. Total organic carbon, total organic nitrogen, stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) and chlorophyll a indicated significant incorporation of organic wastes, increased primary production and trophic niche modifications on the biofilms close to the organic enrichment source. In Spain, where the organic load was higher than in Greece, diatom and, to some extent, bacterial assemblages varied following the organic enrichment gradient. The taxonomic richness of diatom and bacterial communities was not influenced by organic enrichment. Classical community parameters showed consistent patterns to organic pollution in both locations, whereas community assemblages were only influenced when organic pollution was greatest. The successional patterns of these communities were similar to other epilithic communities. The modification of community assemblages induced by organic pollution may affect ecological functions.

1. Introduction

Biological communities typically respond to environmental changes by altering their assemblages. The series of modifications of the assemblages that the biological communities go through due to environmental changes along time or space are known as ecological succession. Ecological succession has been a wide area of study in ecology, especially on terrestrial plant ecology (Clements, 1916; Odum, 1969; Connell and Slatyer, 1977; Tilman, 1990). These studies have led to the development of different models that mechanistically explain how the dominance of different species takes place along succession (Connell and Slatyer, 1977). Anthropogenic disturbances are common drivers that cause environmental changes inducing biological succession (Lake, 2000). The response of the community depends, on the type of disturbance (Houseman et al., 2008).

Organic pollution is one of the most widespread types of pollution in the world (Islam and Tanaka, 2004) and is a main anthropogenic driver of ecological change in marine ecosystems (Halpern et al., 2008). Organic pollution occurs when the input of organic matter increases above natural levels. Although the effects of organic pollution on biological assemblages have been widely studied in macroscopic communities in soft bottoms (Pearson and Rosenberg, 1978), rocky intertidal areas (Roberts et al., 1998) or fouling communities (Cook et al., 2006), microscopic marine communities inhabiting biofilms have received considerably less attention.

Biofilms are mainly composed by microalgae, bacteria, fungi, archea and micrometazooans, embedded in a matrix of extracellular polymeric substances that are secreted by these organisms (Characklis and Marshall, 1990). This matrix is mainly formed of polysaccharides that help the organisms stay attached to the surface and protects them from external disturbances (Hahn et al., 2004; Vu et al., 2009). Biofilms growing on solid surfaces are widespread in aquatic environments (Costerton et al., 1995). Biofilms have key functions in stabilizing sediments (Stal, 2003), providing food for higher trophic levels (Decho and Moriarty, 1990; Abreu et al., 2007; Kuwae et al., 2008), favouring the settlement of marine invertebrate larvae (Crisp and Ryland, 1960) and affecting geochemical cycles and climate change at a global scale (Forney et al., 2004). Biofilms can accumulate pollutants (Morin et al., 2008; Sanz-Lazaro et al., 2011b), which is proven to induce changes in the biological assemblages in freshwater systems

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(Dorigo et al., 2007; Sabater et al., 2007). In marine environments, metal and pesticide pollution alter the species composition of biofilm communities (Magnusson et al., 2012; Licursi and Gomez, 2013), but these effects have been much less studied compared to freshwater systems (Sabater et al., 2007).

Comprehending the changes in biofilm communities related to pollution is a first and necessary step to understand ecological succession and potential changes in ecosystem functioning. Bacterial and microalgae communities of biofilms play a major role in the biogeochemical cycles of elements such as organic carbon and nutrients. Modification of natural assemblages can lead to a decrease on the yield of ecosystem functions that the communities carry out (Bell et al., 2005; Allison and Martiny, 2008; Pholchan et al., 2013). Thus modifications in the bacteria and diatom assemblages produced by organic pollution may influence the performance of the ecological functions of these communities.

In this study we aimed to identify the changes in microalgae and bacterial assemblages along gradients of organic pollution on marine biofilms in the water column. To do so, we examined biofilm communities along an organic pollution gradient in two locations in the Mediterranean (Spain and Greece), that were both influenced by fish farming. We hypothesized that organic pollution would drive changes in microalgae and bacterial assemblages but the successional patterns may not follow the patterns previously studied of other biological communities.

2. Materials and methods

2.1. Study area

The study was conducted in the vicinity of two marine fish farms located in the Mediterranean Sea, which culture gilthead sea bream (*Sparus aurata* L.) and European sea bass (*Dicentrarchus labrax* L.) both with an annual production of ~1000 tons. The first fish farm is located at an open bay, ~500 m from land in Águilas, Murcia, SE Spain (37° 24′ 56″ N, 1° 32′ 4″ W) with a mean bottom current of 7.6 m s^{-1} (Sanz-Lazaro et al., 2011a). The water depth at the area where the net cages are located is 30–35 m. The second fish farm is located in the bay of Sitia, Crete, SE Greece (35° 15′ 18″ N, 26° 13′ 23″ E) about ~50–100 m from land, at a sheltered bay which has a maximum water depth of 20 m. In this fish farm the mean value of the bottom current was 1.8 m s^{-1}. The water depth at the net cages was 14–18 m. While in Spain the fish cages where clustered, in Greece the fish cages were haphazardly distributed along the bay and not all the fish cages were on production (Supplementary material Fig. S1). During the experiment, background levels in the water column of NO_3^- were 4.48 and 3.47 mM for Spain and Greece, respectively, while NO_2^- and PO_4^{3-} levels in both locations were below 0.5 μM. During the time of the experiment, sea surface temperatures were 17–18 and 19–20 °C for Spain and Greece, respectively. In both locations there are no rivers, nor other anthropogenic point-source inputs, thereby fish farms are expected to be a major source of organic matter.

2.2. Experimental design

We used microscope glass slides as the artificial surface for biofilm growth, in order to avoid confounding factors due to possible differences among natural substrates on sampling sites (Webster and Negri, 2006; Sanz-Lazaro et al., 2011b). Glass is a suitable artificial substrate since biofilms growing on this surface are similar to biofilms growing in natural substrates (Jackson et al., 2001). Glass slides were kept vertical by slide holders, which were maintained 3 m below the water surface by an anchoring system and a buoy (see Fig. S2 in supplementary material). Glass slides served as replicates for the different parameters measured once the slides were retrieved.

Two different locations with different natural environmental conditions were chosen to overcome possible biases due to environmental conditions of a specific location. At both locations, glass slides were placed at different distances from the fish farm in an effort to embrace the whole range of organic enrichment caused by fish farming. In Greece, the positioning of the glass slides did not follow a linear transect since fish cages were haphazardly placed on the bay. The distances from a fish cage of the fish farm where the slides were deployed were 0, 5, 20, 60 and 350 m for Spain, while in Greece the range of distance from a fish cage range from 0 to 100 m. Due to the small size of the bay, the range from a fish cage was lower in order to avoid confounding factors due to the natural environmental differences outside this sheltered bay. In Autumn 2009, glass slides were deployed at both locations for 14 days starting the 20th and the 29th of October, in Spain and Greece, respectively. The number of replicates that were analysed for each parameter was 5, whereas for bacterial community analysis it was 3. In some sampling sites few glass slides were broken and thus diatom analysis for those sites was performed on less replicates: one replicate was missing at one sampling site in Spain, and one and two replicates were missing in two sampling sites in Greece.

The biofilm parameters estimated were dry weight biomass, polysaccharides, TOC, TON, stable isotopes ($\delta^{13}C$ and $\delta^{15}N$), chlorophyll *α*, as well as microalgae and bacterial community structure changes.

After the retrieval of the slides, the slides for the DNA extraction analyses were immediately scraped off with a new sterile surgical blade (Surgeon Carbon Steel, Jai Surgicals Ltd.; Haryana, India) for every slide. Biofilm replicates were transferred to individual vials and frozen in liquid nitrogen until laboratory arrival, where they were kept at −80 °C. The biofilm slides that were going to be used for the characterization of the main groups of the biofilm communities were fixed in 4% paraformaldehyde suspended in phosphate buffered saline solution. Once in the laboratory, they were kept at 4 °C. For the rest of the analyses, biofilm samples where kept fresh until laboratory arrival were they were stored at −20 °C, and, before each analysis, the biofilm community was scraped off with a new scraper for every slide. The biofilm slides used for chlorophyll-*α* analysis were kept in the dark immediately after they were collected.

2.3. Microscopic analysis

The characterization of the main biological groups of the biofilm communities was performed by means of a fluorescence microscope (Leica DM6000B; Wetzlar, Germany). First, the day following the retrieval of the glass slides, the biofilm slides that had been fixed in paraformaldehyde were dehydrated by immersing biofilm slides for 5 min on baths of increasing concentrations of ethanol (50, 75 and 96%) and were left to completely dry in air. Then, they were stored in the dark at 4 °C until further processing. Afterwards, the biofilm slides were dyed using the fluorescent DNA-specific stain 4′,6-diamidino-2-phenylindole (DAPI; Sigma, St. Louis, USA) with a concentration of 5 μg ml^{-1} for 10 min. Afterwards, the cover slips were mounted and the biolfilm organisms were visualized on the fluorescence microscope using an excitation filter between 340 and 380 nm and a blue/cyan emission filter of 425 nm. The lenses that we used gave a final magnification between 200 and 1000×. This preliminary analysis showed that diatoms were the predominant biological group among microalgae in both locations. Therefore, we chose the diatom community as a surrogate of the whole microalgae community.