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Polycyclic aromatic hydrocarbons alter the structure of oceanic and oligotrophic microbial food webs

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ABSTRACT

One way organic pollutants reach remote oceanic regions is by atmospheric transport. During the Malaspina-2010 expedition, across the Atlantic, Indian, and Pacific Oceans, we analyzed the polycyclic aromatic hydrocarbon (PAH) effects on oceanic microbial food webs. We performed perturbation experiments adding PAHs to classic dilution experiments. The phytoplankton growth rates were reduced by more than 5 times, being *Prochlorococcus* spp. the most affected. 62% of the experiments showed a reduction in the grazing rates due to the presence of PAHs. For the remaining experiments, grazing usually increased likely due to cascading effects. We identified changes in the slope of the relation between the growth rate and the dilution fraction induced by the pollutants, moving from no grazing to V-shape, or to negative slope, indicative of grazing increase by cascade effects and alterations of the grazers' activity structure. Our perturbation experiments indicate that PAHs could influence the structure oceanic food-webs structure.

1. Introduction

The number of synthetic organic substances has increased enormously during the second half of the twentieth century (Dachs and Méjanelle, 2010), due to increased anthropogenic population growth and activities. PAHs (polycyclic aromatic hydrocarbons) are found in the environment as complex mixtures of different chemicals (Hylland, 2006) and are particularly important due to their toxicity to organisms. There are different emission sources of PAHs to the environment. Catastrophic crude oil spills represent a small fraction of the total crude oil discharge into the sea (NRC, 2003). The PAHs are transported long distances through the atmosphere, reaching remote oceanic areas where they are deposited (Del Vento and Dachs, 2007). Warm and oligotrophic waters characterize the large open central areas of the Atlantic, Indian and Pacific oceans where phytoplankton biomass and primary production are dominated by picophytoplankton (Agawin et al., 2000). The picophytoplankton is mainly composed by cyanobacteria *Synechococcus* sp. and *Prochlorococcus* sp., and small picoeukaryotic organisms, which are rarely classified taxonomically (Campbell et al., 1997; Li et al., 1992). These species play an important role in the primary production at the global scale, as these oligotrophic waters represent 70% of the oceans surface.

The PAH toxicity effects for aquatic organisms are well known (Lehr and Jerina, 1977). In phytoplankton they can generate changes in cellular processes, in photosynthesis, pigments composition and growth rates (e.g. Singh and Gaur, 1988). PAHs are hydrophobic substances, and once they reach the water column they tend to accumulate in planktonic organisms, and are then, transfer to higher organisms through the trophic chain (Dachs et al., 2002; Echeveste et al., 2010a).

Previous studies have examined the effect of persistent organic pollutants in phytoplankton communities, and have identified negative effects depending on the species cell size (Echeveste et al., 2010a). The picophytoplankton was described as the most sensitive organisms within the phytoplankton communities to these pollutants, particularly *Prochlorococcus* sp. and *Synechococcus* sp., due to their small size (0.1 μm^3 and 0.9 μm^3 respectively). Small microorganisms dominate the food webs in the oligotrophic oceans. The autotrophic picophytoplankton dominates the phytoplankton biomass, being the first step in the food web (Agawin et al., 2000; Li et al., 1992). Their small size determines the low size of their predators. The picophytoplankton is grazed by protists and zooplankton organisms such as ciliates, dinoflagellates and heterotrophic nano-flagellates (e.g. Christaki et al., 1999). These organisms are responsible for 50 to 100% of grazing rates of oceanic primary producers (Calbet and Landry, 2004; Schmoker et al., 2013).

PAHs tend to bioaccumulate (Gerofke et al., 2005) and are transferred through marine food webs, incorporating PAHs from zooplankton organisms by grazing on phytoplankton, and from the water column through diffusive process (Berrojalbiz et al., 2009). PAHs may also affect the grazing efficiency. Previous works has described different

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pollutants effects on grazing and growth rates of preys. For example, Sibley et al. (Sibley et al., 2004) showed, using mesocosms experiments, that the presence of creosote (complex mixture made up of 85% PAHs), had negative effect on zooplankton grazing rates. It was observed that the phytoplankton abundance increased as a result of the decreasing in the grazing pressure. To our knowledge, there has been no attempt yet to quantify how PAHs influence the grazing on primary producers by herbivores, in oceanic microbial food webs.

Mimicking the microbial oceanic food webs and the oligotrophic conditions in the laboratory is difficult, and has so far prevented the advance in resolving how pollution may affect phytoplankton and zooplankton relationships in the oceanic systems. Previous studies, in coastal areas using mesocosms, have shown that zooplankton are more sensitive to PAHs than phytoplankton (Sibley et al., 2004; Medina et al., 2004), so we hypothesize that the balance between growth and losses of phytoplankton of the open oceanic areas could be strongly affected by PAHs. The rational for this work was to investigate the effect of PAHs in the interaction between phytoplankton and its grazers in natural planktonic communities in the oligotrophic open-ocean, from the Atlantic, Indian and Pacific Oceans, during the Malaspina-2010 oceanographic circumnavigation expedition. We conducted dilution experiments with the aim to analyze the effect of PAHs in the balance between the grazing and growth rates into the planktonic food webs (Landry and Hassett, 1982). We followed a modification by Landry et al. (Landry et al., 1995). It is the most widely method utilized to estimate "in situ" the grazing rates of microzooplankton. Perturbation experiments were conducted by adding different concentrations of a PAH mixture to natural planktonic communities, to test how PAHs can disrupt the grazing/growth rates of planktonic communities in oligotrophic oceanic systems.

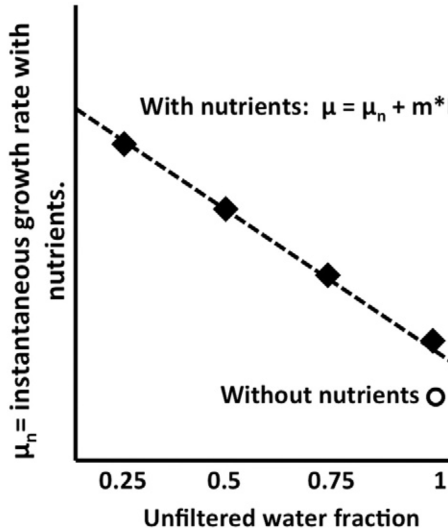

Fig. 2. Dilution model. Linear regression between the unfiltered water fraction and the instantaneous growth rate in the dilution series with nutrients, following Landry et al. (1995). μ_n represents the instantaneous growth rate for each dilution; μ represent the growth rate; m corresponds to the grazing rate.

2. Methods

Experiments were conducted on board the Spanish R/V BIO Hespérides during the Malaspina-2010 circumnavigation expedition, which performed 7 oceanographic cruises through the Atlantic, Indian and Pacific oceans from December 2010 to July 2011 (Fig. 1. Map made by ESRI ArcGIS Online).

Water samples for dilution experiments were collected from 3 m depth using 30 L Niskin bottles. Water temperatures at sampling depth were measured by sensors installed in the continuous water flow system of the vessel.

2.1. Experimental design

Growth and grazing rates were determined using the modified dilution technique of Landry et al. (1995), based on the consumption of the prey in situ, allowing quantification of the phytoplankton growth and grazing mortality rates (Landry and Hassett, 1982). This technique consists of incubating different dilutions of water samples diluted with filtered seawater to different degrees of dilution (Fig. 2), since progressive dilutions are assumed to suffer progressively less grazing impact. For each diluted fraction it was estimated the instantaneous phytoplankton net growth rate (μn, Fig. 2), from changes in chlorophyll *a* concentration (Chl*a*) or in the abundance of the phytoplankton population. The estimates of grazing mortality (m) and growth rates (μ) were calculated as the *y*-intercept and slope of the linear regression of observed net growth rate against experimental dilution (ranging between 0 and 1; Fig. 2). To test the effect of the PAHs on the relationship between grazing and growth rates on phytoplankton community, we run 19 experiments across all oceans (Fig. 1), consisting on three different dilution series: a control without pollutants, and two series of dilutions to which we added two different concentrations (ranging from 0.1 to 3.6 μg L^{-1}; Table 1) of a PAHs mixture.

Four different dilutions were employed in each series, corresponding to unfiltered seawater fractions of 0.25, 0.50, 0.75, and 1. The dilutions consisted in adding 0.2 μm filtered seawater to the samples, applying the gravity filtration through capsule filters (0.2 μm pore, Pall Corporation). The filtrate volume for each dilution fraction was

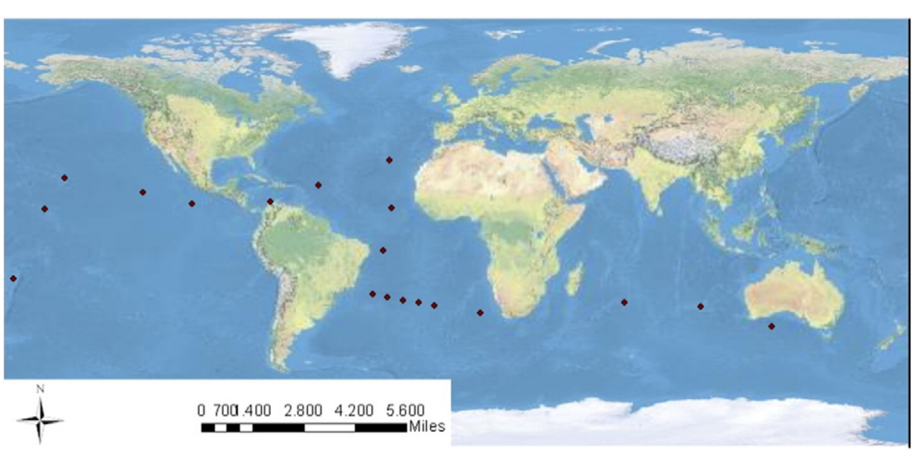

Fig. 1. Study area. Position of the stations sampled for the dilution experiments in the Atlantic, Pacific and Indian Oceans during the study along the Malaspina-2010 expedition.