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Factors influencing the microplastic contamination of bivalves from the French Atlantic coast: Location, season and/or mode of life?

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ABSTRACT

Monitoring the presence of microplastics (MP) in marine organisms is currently of high importance. This paper presents the qualitative and quantitative MP contamination of two bivalves from the French Atlantic coasts: the blue mussel (*Mytilus edulis*) and the Pacific oyster (*Crassostrea gigas*). Three factors potentially influencing the contamination were investigated by collecting at different sampling sites and different seasons, organisms both wild and cultivated. Inter- and intra-species comparisons were also achieved. MP quantity in organisms was evaluated at 0.61 ± 0.56 and 2.1 ± 1.7 MP per individual respectively for mussels and oysters. Eight different polymers were identified. Most of the MPs were fragments; about a half of MPs were grey colored and a half with a size ranging from 50 to 100 µm for both studied species. Some inter-specific differences were found but no evidence for sampling site, season or mode of life effect was highlighted.

1. Introduction

Plastics have changed our life since their appearance in the middle of the last century (Thompson et al., 2009). They are massively used as they replaced traditional materials due to their multiple advantages. Actually, their worldwide production is increasing, reaching > 300 million tons last years (PlasticsEurope, 2015). In contrast, many damages by plastic wastes to ecosystems were observed and reported (Cole et al., 2011; Gall and Thompson, 2015) due to lacks in plastic waste management faced to the increase of plastic uses. A recent work of Jambeck et al. (2015) estimated that approximately 4.8 to 12.7 million tons of plastic wastes entered the ocean only in 2010.

Besides, plastic wastes are known as the main source of microplastics (MPs) which were defined as plastics with a size inferior to 5 mm by Arthur et al. (2009). The fragmentation of plastic wastes occurring in the environment and leading to MPs is due to mechanical, chemical and biological factors (Andrady, 2011; Costa et al., 2010; Zettler et al., 2013) and corresponds to the secondary source of contamination. The primary contamination of MPs is characterized by the microspheres used in the industry, in the personal care products for example. Because of their small size and their properties, MPs can be accumulated in the environment leading to a potential MP exposure of biota undergoing bioaccumulation and biological effects (Barnes et al., 2009; Wright et al., 2013). The occurrence of MPs was reported in continental environments: air (Gasperi et al., 2015), freshwater (Dris et al., 2015b; Free et al., 2014; Jambeck et al., 2015), wastes and treated waters (Dris et al., 2015a), lake sediments (Fischer et al., 2016) and soil organisms (Huerta Lwanga et al., 2016; Rillig, 2012). Numerous studies focused also on marine environments, studying seawater (Cózar et al., 2015; Desforges et al., 2014; Van Cauwenberghe et al., 2015); sand and sediment (Blaskovic et al., 2017; Graca et al., 2017; Woodall et al., 2014) and many marine animals (Besseling et al., 2015; Desforges et al., 2015; Karami et al., 2017). In marine ecosystems, many species of invertebrates are known to accumulate contaminants from seawater and marine sediments, representing valuable biological indicators of environmental pollution (Kaiser, 2001). Among these species, benthic suspension-feeding bivalves, such as mussels or oysters, have been widely used in biomonitoring surveys in coastal waters (e.g. the Mussel Watch program; Farrington et al., 1995; RNO, 2006), on account of their abundance and broad distribution, their suspended particle filter-feeding mode, their sedentary living and their importance for human food web. As for chemical hazards, these species may be good indicators of MP contamination in the water column (Avio et al., 2017). Published studies presenting data on MP contamination in marine bivalves are scarce at this moment even if many of them are currently performed. Comparisons are furthermore debatable because

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of a lack of standardized protocols (Phuong et al., 2016).

In this context, the present study aimed to evaluate the MP contents of two filter-feeder bivalve species, the blue mussel, *Mytilus edulis* and the Pacific oyster, *Crassostrea gigas*, collected on the French Atlantic coast (Pays de la Loire, France) according to a validated protocol (Phuong et al., 2016). Mussels and oysters have a considerable socio-economic weight in this regional area where 16 thousand tons are annually produced for national consumption. For both species, several sampling sites, seasons and modes of life (cultivated and wild) were investigated in order to determine the factors influencing the contamination and to run an inter-species comparison. The seasonality was evaluated through two sampling campaigns that were performed in autumn and spring, selected because these periods arise from times of high exposure of organisms. The beginning of autumn succeeds to summer which is a period of strong anthropic pressure due to tourism activities on the coast and it is after a warm period leading to higher rate of filtration of suspensivorous bivalves. Moreover, this time is included in the main commercialization periods of mussels (summer and autumn) and oysters (autumn and winter). The beginning of spring arises from the winter period marked by stormy climatic conditions which remobilize the contaminants stored in the sediments, increasing the risk of organism exposure.

Furthermore, the relation between MP contamination and the living environment of organisms was suggested by previous studies (Mathalon and Hill, 2014; Van Cauwenberghe and Janssen, 2014; Vandermeersch et al., 2015; Li et al., 2016). For example, Li et al. (2016) found significant differences between mussels sampling along about 20,000 km coastline showing that sampling location could be one important factor influencing MP content in marine ecosystems. The sampling sites chosen in this study are separated of 140 km at the maximum. They were selected because of the intensive and important shellfish aquaculture for regional and national socio-economy and because they are under different oceanic and terrigenous influences. Moreover, wild mussels were shown to be more contaminated than farmed ones in this latter study, while the opposite was found by Mathalon and Hill (2014). As numerous tools made of plastic are used in mussel and oyster farms for spat collection or on-growing stages, wild and cultivated organisms from different major farming areas along the Atlantic coast were analyzed in the present work to investigate the influence of farming practices on MP contents, and assess the spatial variations of MP contamination. Overall, it provides first data on the MP contamination level of the French Atlantic coast for these two chosen bivalve species, contributing to the global MP contamination assessment.

2. Materials and methods

2.1. Reagents

KOH pellets were purchased from CARLOERBA reagents (France); KI pellets were from VWR (France) and the membrane filters of cellulose nitrate with pores of 12 μm and 25 mm of diameter were provided by Whatman (Germany).

2.2. Sample collection strategy

Fig. 1 shows the sampling locations of adult mussels (*M. edulis*) and oysters (*C. gigas*) on French Atlantic coasts (Pays de la Loire region, France). Oysters were sampled in the three points located on the map: Pen-Bé (N 47°25′33″ W 2°27′46″), Coupelasse (N 47°01′19″ W 2°01′59″) and Aiguillon Bay (N 46°16′26″ W 1°14′14″) while mussels were only sampled in Pen-Bé and Aiguillon Bay because of their absence in the third site. For each sampling site, wild and cultivated organisms were randomly collected at two different seasons: October 2015 (beginning of autumn) and March 2016 (beginning of spring).

The sampling site Pen-Bé is located in the Pen-Bé bay. The watershed of the bay is mainly represented by agriculture activities (60%) and urbanization (15%) (IFREMER, 2006). The urbanization is quite limited since the towns present in the watershed do not have > 2000 inhabitants. Activities of salt production are also present in the watershed (580 ha) as well as the aquaculture of mussels and oysters (280 ha). The supply of freshwater of the bay is limited. The impacts of the Loire estuary are negligible whereas those of the estuary of the Vilaine can be considered depending on weather conditions. In the bay, it was measured that about 41 to 92% of the water is renewed after 5 tidal cycles. The samples were collected at about 300 m of the coastline.

Coupelasse is located in the bay of Bourgneuf (340 km^2). The watershed is mainly represented by protected zones (habitats and birds), in particular in the south where the marsh Breton is recognized for its biodiversity as 3rd national wetland (IFREMER, 1997). The main supply of freshwater is done by Millac, a small river draining a watershed composed of hedgerows, grasslands and 2 towns in its downstream part (Les Moutiers en Retz and Bourneuf en Retz: 1500 inhabitants and 3500 inhabitants respectively) (Schéma d'aménagement et de gestion des eaux du marais breton et du bassin versant de la baie de Bourgneuf, 2014). It is the main shellfish farming area in the Pays de la Loire region with high oyster farming activity (90%) and little mussel farming explaining why no mussels were sampled for this study. This area is separated from the ocean by the island of Noirmoutier which shelters it from the swell. It communicates with the ocean to the north through an opening of 12 km. The oceanic waters more salted than the waters of Bourgneuf lead to a difficult mixing of them. It was estimated that it takes about 2 months for the waters of the Bay of Bourgneuf to be renewed by ocean waters. The bay is to the south of the Loire estuary but its plume rarely penetrates the bay only by northerly wind or in low water period. The samples were collected at about 1 km of the coastline.

The samples taken in Aiguillon bay were collected just outside the bay to the north, remaining in the Pays de la Loire region. This site is exposed offshore and is under oceanic and terrigenous influences (IFREMER, 1996). The terrigenous influence is limited and comes from the bay where the residence times of the waters appear to be quite long and the exchanges relatively limited with the outside. Freshwater supply of the bay is due to the rivers Sèvre Niortaise and the Lay and less frequently to the Canal du Curé. All these rivers drain the watershed of the Poitevin marsh which is a protected zone. Along the coast, an extensive mussel farming activity occurs accounting for 15% of the national production. The samples were collected at about 300 m of the coastline.

Five samples, i.e. 5 oysters and 5 pools of 3 mussels, were analyzed per sampling location, per season and per mode of life, leading to a total of 180 marine bivalves: 60 oysters and 120 mussels.

2.3. Biometric parameters and sample preparation

After the collection, organisms were placed in sealed freezer plastic bags made of PE and conducted to the laboratory in a refrigerated enclosure. Organisms were then kept in bags at − 20 °C until analysis. All of laboratory experiments were carefully performed with the aim of preventing MP contamination. Concerning sample preparation, the bivalves were taken out of the freezer for thawing during 1 h. Then, each oyster and mussel shell was individually measured and the individual total wet weight without internal water and soft tissue wet weight were determined after the elimination of the byssus for the mussels. Organisms sized from 3.5 to 5.8 cm and from 7.3 and 13.7 cm for mussels and oysters respectively. The condition index (CI) was calculated according to the recommendation of the French Association for Standardization (AFNOR, 1985) using the formula: CI = (soft tissue wet weight / total wet weight without internal water) × 100. CI ranged from 22.3 to 53.5 for mussels and from 17 to 46.8 for oysters.

MP analyses were performed on individual oysters and on a pool of 3 mussels following the protocol elaborated by Phuong et al. (2017). In brief, the sample (one oyster or a pool of 3 mussels) was placed into a