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Persistent and emerging pollutants assessment on aquaculture oysters (*Crassostrea gigas*) from NW Portuguese coast (Ria De Aveiro)

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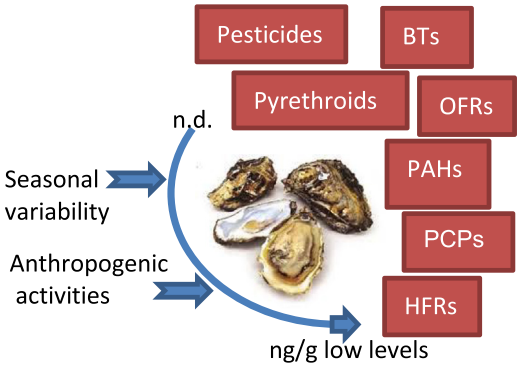
HIGHLIGHTS

- Bioaccumulation of organic contaminants assessed in aquaculture farmed oysters.
- Emerging and persistent organic contaminants detected, generally at low levels
- Most frequently detected families were halogenated flame retardants and personal care products.
- Seasonal variations related with human activities were detected.
- Results highlights the need for environmental protection and sustainable resource exploration.

GRAPHICAL ABSTRACT



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ABSTRACT

The study aim was to determine a range of relevant persistent and emerging pollutants in oysters produced in an aquaculture facility located in an important production area, to assure their safety for human consumption. Pollutants, including 16 PAHs, 3 butyltins (BTs), 29 flame retardants (FRs, including organophosphate and halogenated FRs), 35 pesticides (including 9 pyrethroid insecticides) and 13 personal care products (PCPs, including musks and UV filters), were determined in oysters' tissues collected during one year in four seasonal sampling surveys. The seasonal environmental pollution on the production site was evaluated by water and sediment analysis. Furthermore, oysters' nutritional quality was also assessed and related with the consumption of healthy seafood, showing that oysters are a rich source of protein with low fat content and with a high quality index all year around. Results showed that most analysed pollutants were not detected either in oyster tissues or in environmental matrixes (water and sediments). The few pollutants detected in oyster tissues, including both regulated and non-legislated pollutants, such as a few PAHs (fluorene, phenanthrene, anthracene, fluoranthene, pyrene

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Nutritional quality
Oysters' safety

and indenopyrene), FRs (TPPO, TDCPP, DCP, BDE-47, BDE-209 and Dec 602) and PCPs (galaxolide, galaxolidone, homosalate and octocrylene), were present at low levels (in the ng/g dw range) and did not represent a significant health risk to humans. The observed seasonal variations related to human activities (e.g. tourism in summer) highlights the need for environmental protection and sustainable resource exploration for safe seafood production.

1. Introduction

Aquaculture is one of the fastest-growing food-producing sectors worldwide (FAO, 2016), with an annually growth around 2% (The EU Fish Market, 2018). Oysters, despite not being at the top of the most traded and consumed seafood items, are among the five most valued species farmed in the EU (The EU Fish Market, 2018).

Oyster farms can be found in estuarine areas, taking advantage of their natural resources. Estuaries have high biological productivity, but as they are close to populated areas, they are also exposed to potentially polluting activities, which can negatively impact these areas and affect their important ecological role (Granek et al., 2016). So, in estuarine environments, the exposure of oysters to pollutants can pose a risk to their production in aquaculture systems and potentially to consumers' health.

The presence of contaminants in the environment can indeed affect the development of organisms. For instance, oysters have shown to be negatively affected (e.g. oxidative stress) by the presence of metals and polycyclic aromatic hydrocarbons (PAHs) in water (Toledo-Ibarra et al., 2016) and abnormal development (e.g., decrease in growth and shell size) due to the accumulation of PAH compounds (Sarker et al., 2018; Nogueira et al., 2017; Geffard et al., 2001). Oysters ingest suspended particles by filtering the surrounding water and may bioaccumulate pollutants present in the water column (Munksgaard et al., 2017). In fact, oysters have been used as sentinel species to assess ecosystem health disturbance and for pollution monitoring (Aguirre-Rubí et al., 2018) and have been frequently used to monitor metal and pesticides pollution in estuarine waters (Rodriguez-Iruretagoiena et al., 2016; Raub et al., 2015).

Seafood is mostly recognized as a high-quality and healthy item, but it can also be a source of harmful environmental contaminants (ECR 1881/2006, 2006 amended by ECR, 2008, 2011a, 2011b). Toxic contaminants present in seafood may pose risks to human health, through the ingestion of contaminated seafood.

For that reason, maximum concentrations were established by the European Commission for several contaminants in seafood, e.g. metals, such as Cd, Pb and Hg, dioxins, PCBs or PAHs (ECR 1881/2006, 2006 amended by ECR, 2008, 2011a, 2011b). Moreover, monitoring programs are regularly carried out for regulated contaminants to guarantee that seafood is safe for human consumption (Vandermeersch et al., 2015). Yet, a wide range of emerging and persistent contaminants are still not studied, and maximum values are still not defined in the European legislation due to the lack of sufficient information. The concern with several of these compounds is increasing because their persistence, bioactivity and bioaccumulation potential can cause significant harmful effects both on ecosystems and on human health. The lack of information regarding their presence in seafood represents a potential risk. In this way, more contaminants should be assessed in different seafood items, including those produced in farms established in coastal zones subjected to significant anthropogenic pressures.

The oysters produced in Portugal occupy a relevant place in the southern European markets, being a widely appreciated seafood item. The most common oyster species produced in Portugal is *Crassostrea gigas*, a species well distributed around the world. In Portugal, one of the oyster farming sites is located in Ria de Aveiro, NW Portugal, where *C. gigas* is extensively produced. However, a wide range of studies carried out in Ria de Aveiro indicate the presence of pollutants in several areas of this region (Pereira et al., 2009). So, aquacultures situated in Aveiro need to be regularly monitored to assess its environmental status. Some contaminants, such as PAHs, have been determined in oysters (Sarker et al., 2018; Nogueira et al., 2017; Geffard et al., 2001), but, to our knowledge, no studies on personal care products (PPCs) accumulation in these organisms have been carried out.

The aim of the present work was to determine, over a seasonal cycle, a wide range of persistent and emerging contaminants in oysters produced in a farm in Ria de Aveiro to guarantee seafood safety to the final consumer. Pollutants, including PAHs, butyltins (BTs), flame retardants (FRs), pesticides and pharmaceuticals and personal care products (PPCPs), were measured in oysters' tissues collected over one year in four sampling surveys, as well as in water and sediment within the production site to evaluate the pollution load and its possible seasonal variations. Highly dynamic interactions between sediments/water/organisms in estuaries may be affected by seasonality and pollutants accumulation can vary with time (Granek et al., 2016). Data on water and sediments provides indication of possible pollution sources that could pose a risk to aquaculture farms. The nutritional value of all collected oysters was also assessed to evaluate possible environmental seasonal influence in oyster quality and its relation with oysters' safety. As far as we know the pattern of quality and safety of oysters commercially exploited in Portugal was never evaluated within a production area over time, thus our data may contribute to enhance the market value of oysters produced in Ria de Aveiro.

2. Material and methods

2.1. Sampling area

The sampling area is located in the western Portuguese Coast (Aveiro), in a region very relevant for aquaculture production named Canal de Mira (Fig. 1).

Ria de Aveiro is a unique ecosystem. The "Rias" were formed a couple of centuries ago when seawater advanced to land, leading to the formation of lagoons, becoming a unique natural landscape in the Iberian coast (Ramalhosa et al., 2005).

According to the European Commission (2006), Aveiro is situated in "fisheries-dependent communities". Aveiro has shipyards for maintenance and shipbuilding, cold storage, manufacture and maintenance of fishing gears, contributing significantly to the local economy, but also contributing to an increasing anthropogenic pressure in this region. Ria de Aveiro is, therefore, under several anthropogenic pressures and suffers the influence of a wide range of environmental pollutants, including metals and persistent organic pollutants such as PAHs (Gadelha et al., 2011). Moreover, treated/untreated sewage discharge can also be a major pollutant pathway into this environment. The treated sewage of the whole Aveiro lagoon (325,700 population equivalent) is collected and discharged 3.3 km offshore. Treatment and/or collection of effluents have been reported as only partial (Rada et al., 2016).

The sampling station was located in Canal de Mira arm of Ria de Aveiro (40.61721N; −8.74408 W) that receives seawater intrusion,

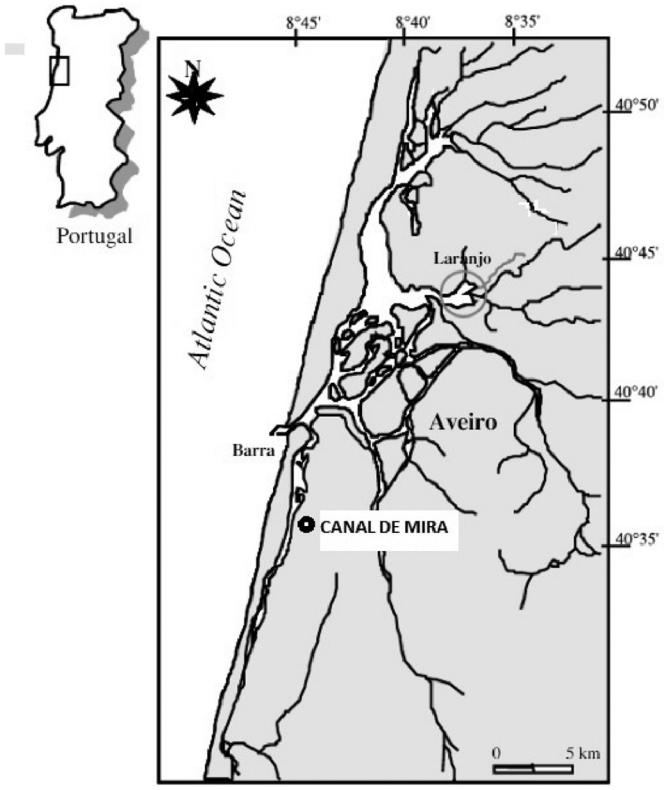

Fig. 1. Schematic figure representing the Ria de Aveiro with indication of the sampling site at Canal de Mira.
(Source: Adapted from Abreu et al., 2010).

but also inland drainage from agriculture fields within the premises of the oyster production farming area.

2.2. Sampling procedures

Sampling was carried out during a complete seasonal cycle: Summer (S1), Autumn (S2) Winter (S3) and Spring (S4), respectively in July 2016, November 2016, January 2017 and May 2017, at rising high tide.

Thirty-five oysters of "commercial size" (gauge T3 – 66–86 g total weight) were randomly collected in each season by hand, directly from the aquaculture bags which are cultured on trays. At the same location, water and sediment were also collected. Physical and chemical parameters, namely temperature, salinity, pH, oxygen saturation and turbidity were measured in situ using a YSI6920 CTD multiparameter probe (YSI Inc., Yellow Springs, OH, USA). Surface water samples were collected using 500 ml acid-cleaned polyethylene or glass bottles depending on the contaminants, or physical-chemical parameter being analysed. Sediment samples were collected with a dredge (Petit Ponar, Widco), with a 15 cm × 15 cm opening, corresponding to a 6.75 l of sediment, operated from a boat. Each time, triplicates samples were pooled, homogenized and stored in air-tight polyethylene bags. All samples were kept in the dark and refrigerated in ice chests before transportation to the laboratory.

The thirty-five oysters collected at each season were taken to the laboratory and biometric data were recorded individually (total weight and total length). The oysters were divided in two groups: one for contaminants determination (n = 20) and other for nutritional quality (n = 15). For contaminants assessment, oysters' tissue and intervalvar liquid were collected and total weights were determined (tissue weight, n = 20). The soft tissues together with the intervalvar liquid were freeze-dried, grounded and divided in five pools for analysis of several groups of chemical compounds. For nutritional studies, the soft tissue of each individual was weighted, snap-frozen and kept at −80 °C before analysis. Then, each group of fifteen oysters was slightly defrosted for grinding and the homogenized mixture was divided in five pools (5 pools/season, total of 20 pools).

Water samples from each season were filtered: a) through 0.45 μm pore size acetate cellulose filters and kept at −20 °C until analysis, for contaminants assessment; b) through glass microfiber GF/F filters (Whatman, GE), for particulate matter (PM), particulate organic matter (POM) determination, carbon (total dissolved carbon, organic dissolved carbon and inorganic dissolved carbon) and total nitrogen measurements; and c) through 0.45 μm nitrocellulose membrane filters (Merck Millipore) for assessment of nutrient concentrations (nitrate, nitrite, ammonium and phosphate ions).

With regard to sediments, for contaminants assessment, sediment samples from each season were firstly freeze dried. Afterwards, samples were grounded and stored in the dark at room temperature until analysis. For physical-chemical characterisation of sediments, samples were dried at 100 °C.

2.3. Nutritional value

Chemical analyses were run in duplicate, following AOAC procedures. Moisture was determined in the fresh pooled samples after drying at 105 °C for 24 h. Afterwards, pooled samples of oysters were freeze-dried for the determination of: ash content by incineration in a muffle furnace at 500 °C for 6 h (Nabertherm L9/11/B170, Germany); crude protein (N × 6.25) by a flash combustion technique followed by a gas chromatographic separation and thermal conductivity detection (LECO FP428, USA); lipid content by petroleum ether extraction using a Soxtherm Multistat/SX PC (Gerhardt, Germany) and gross energy in an adiabatic bomb calorimeter (IKA C2000, Germany). Glycogen content was determined in duplicate in the freeze-dried pool samples, according to the method described by Viles and Silverman (1949). Briefly, 25 mg of freeze-dried oyster tissue were hydrolysed with 15 ml of 33% potassium hydroxide in a water bath at 100 °C during 15 min. The absorbance was measured at 620 nm, using a calibration curve prepared with oyster glycogen standard (Sigma). The anthrone solution was prepared at a final concentration of 1.41 mg/ml, using concentrated sulphuric acid.

2.4. Water physical-chemical parameters

Nutrients concentration (phosphate anion (PO_4^{3-}), nitrite anion (NO_2^-), nitrate anion (NO_3^-), ammonium cation (NH_4^+)) were determined colorimetrically using methods described in Grasshoff et al. (1983), and chlorophylls (Chla) concentration was assayed according Parsons et al. (1984). Particulate organic matter (POM) was measured as percentage of weight loss on ignition (500 °C, 2 h) (APHA et al., 1992). Determination of dissolved total carbon (TC), organic carbon (DOC) and total nitrogen (TN) was performed by high temperature catalytic oxidation with a TOC-VCSN analyser coupled to a total nitrogen-measuring unit (Shimadzu Instruments) according to Magalhães et al. (2008). For each sample, all analyses were carried out in triplicate.

2.5. Sediment physical-chemical characteristics

Organic matter content in sediments (each sample analysed in triplicate) was estimated by loss on ignition (4 h at 500 °C), in sediments previously dried at 100 °C. Grain size analysis was performed by sieving samples previously dried at 100 °C, in a mechanical shaker (Percival and Lindsay, 1997). The adopted standard system for particle size limits was the followed: silt and clay (<0.063 mm), fine sand (0.063–0.25 mm), medium sand (0.25–1 mm), coarse sand (1–2 mm), and gravel (>2 mm). Each fraction was weight and expressed as percentage of the total dry weight.

2.6. Chemical contaminants

Several chemical contaminants were determined in water, sediment and oyster tissue samples as described in the following sections. Three replicates were analysed per sample.

2.6.1. Polycyclic aromatic hydrocarbons (PAHs)

The sixteen United States Environmental Protection Agency (USEPA) priority polycyclic aromatic hydrocarbons (PAHs), i.e., naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indenopyrene, dibenz(a,h)anthracene and benzo(ghi)perylene were measured in water, sediment and oyster samples, adapting methodologies described in Aminot et al. (2017) and Rocha et al. (2018). Details are presented in Supplementary material S1.

Water samples were extracted by solid phase extraction (SPE) on a 200 mg Waters Oasis HLB sorbent, followed by analysis by gas chromatography with mass spectrometric detection (GC–MS), operating in SIM mode. For sediment samples, sequential ultrasonication assisted extractions, followed by SPE, were carried out before GC–MS analysis. For oyster tissues, PAHs were extracted by salting-out liquid-liquid extraction with acetonitrile, cleaned by dispersive solid phase extraction (d-SPE) and analysed with GC–MS.

The accurate quantification of PAHs was ensured by the use of their deuterated homologue molecule as internal standard. The procedural recoveries were in the range of 72–117% and reproducibility varied between 1 and 18% (Table S1 in supplementary material). Limits of detection and limits of quantification ranged from 0.15 to 4.7 and from 0.5 to 16 ng/g dry weight (dw) for sediments and oyster tissues, respectively. For water samples, values ranged from 0.2 to 15 ng/l.

2.6.2. Butyltins (BTs)

Butyltins (BTs), monobutyltin (MBT), dibutyltin (DBT) and tributyltin (TBT), were measured in water, sediment and biota samples using an adapted procedure described by Carvalho et al. (2007) and Rocha et al. (2018). Details are presented in Supplementary material S1.

BTs were measured by solid-phase microextraction (SPME)-GC–MS, using NaBEt4 as derivatizing agent. A fiber of poly(dimethylsiloxane) (PDMS, Supelco) was used for SPME. Solid samples were previously extracted in an ultrasonic-bath with a concentrated hydrochloric acid/ethanol 1:1 (v/v) solution. For water samples quantification, a calibration curve with BTs aqueous standard solutions was performed every day. In solid matrix, the analytes were always quantified by the standard addition method. Linear response range was from 0.02 to 1250 ng/g for MBT, 0.07–1500 ng/g for DBT and 0.04–2100 ng/g for TBT and reproducibility below 15% was always obtained. Limits of detection were 1.5 μg/l, 2 μg/g dw and 4 μg/g dw for water, sediment and oysters' tissue samples, respectively. Limits of quantification were 5 μg/l, 7 μg/g dw and 13 μg/g dw for water, sediment and oysters' tissue samples, respectively.

2.6.3. Flame retardants (FRs)

2.6.3.1. Organophosphate flame retardants (OPFRs). Fourteen organophosphate flame retardants (OPFRs), including tris(2-butoxyethyl) phosphate (TBOEP), tris(chloroethyl)-phosphate (TCEP), tris (chloroisopropyl)-phosphate (TClPP), trihexyl phosphate (THP), tris (2-ethylhexyl) phosphate (TEHP), isodecyldiphenyl phosphate (IDPP), 2-ethylhexyldiphenyl phosphate (EHDPP), diphenyl cresyl phosphate (DCP), tributyl phosphate (TBP), triphenyl phosphate (TPHP), triphenylphosphine oxide (TPPO), tris(1,3-dichloro-2-propyl)phosphate (TDCPP), tris(monochloropropyl) phosphate (TMCP) and isopropyl phenyl phosphate (IPPP), were analysed in sediment and oyster samples according to Giulivo et al. (2016). Details are presented in Supplementary material S1.

Sediments were extracted by pressurized liquid extraction (PLE), whereas ultrasound extraction was chosen for oyster samples. Then, an online sample purification and analysis was performed with a turbulent flow chromatography (TFC) in combination with liquid chromatography (LC)-tandem mass spectrometry (MS-MS).

For the analysis of OPFRs tris(isobutyl) phosphate (TIBP) and tris (butyl)phosphate (TNBP) in sediments and oyster samples, the methodology described for PAHs was used.

In water samples only TIBP, TNBP, TCEP, TCIPP, TDCPP, TPHP and EHDPP were analysed, using the methodology described for PAHs.

Recoveries between 47 and 108% were obtained, being always within the range of acceptability (40–120%) for analytical methods based on quantification by isotopic dilution, with reproducibility between 1.3 and 12% (Table S2 in supplementary material). Limits of detection and limits of quantification ranged from 0.2 to 19 and from 1.0 to 25 ng/g lipid weight (lw), respectively, for oysters' tissues and from 0.02 to 1.2 and from 0.05 to 3.4 ng/g dw, respectively, for sediments. For water samples, limits of detection and of quantification ranged from 0.1 to 175 and from 0.4 to 584 ng/l.

2.6.3.2. Halogenated flame retardants (HFRs). Halogenated flame retardants (HFRs) including eight polybrominated diphenyl ethers (PBDEs, congeners 28, 47, 99, 100, 153, 154, 183, 209) and five dechloranes (Dec 602, 603, 604, *syn*- and anti- dechlorane Plus (DP)) were assessed only in sediment and oyster samples, following the protocol optimized by Barón et al. (2014). Details are presented in Supplementary material S1. As HFRs have higher octanol-water partition coefficient and low water solubility, it is highly unlikely to detect halogenated compounds in water (Aznar-Alemany et al., 2018), therefore, they were not analysed in water samples.

Extraction of HFRs from sediments and oyster tissues was performed by PLE, being oyster tissues extracts subjected to an acid treatment with concentrated H_2SO_4. Finally, extracts were purified with SPE using alumina cartridges (AL-N, 5 g). Analysis was carried out by GC–MS-MS.

Recoveries between 59 and 114% were obtained, with reproducibility between 1.3 and 11% (Table S2 in supplementary material). Limits of detection and limits of quantification ranged from 0.007 to 3, and from 0.02 to 10 ng/g lw, respectively, for oysters' tissues and from 0.0001 to 1.7, and from 0.0003 to 5.5 ng/g dw, respectively, for sediments.

2.6.4. Pharmaceuticals and personal care products (PPCPs)

2.6.4.1. Pharmaceuticals. Twenty-five pharmaceuticals were measured in water and sediments. No analyses in oyster tissues were carried out as no validated analytical methodology was available in the laboratory. However, determination of the presence of these contaminants in the environment can contribute for assessment of a potential exposure risk of oysters.

Non-psychiatric pharmaceuticals including paracetamol, cimetidine, atenolol, phenazone, ketoprofen, bezafibrate, budesonide and fenofibrate were extracted from water using SPE according to a modified protocol (developed by Nannou et al., 2015). Psychiatric pharmaceuticals including olanzapine, amisulpride, mirtazapine, carbamazepine, venlafaxine, bupropion, quetiapine, risperidone, clozapine, alprazolam, diazepam, citalopram, haloperidol, amitriptyline, paroxetine, fluoxetine, sertraline were extracted from water according to a protocol developed by Silva et al. (2014).

For sediment samples, all pharmaceuticals mentioned above were extracted according to a modified QuEChERS approach (Nannou et al., 2019). Details are presented in Supplementary material S1. Analysis was carried out by HPLC-MS. To allow the accurate quantification of the different pharmaceuticals, appropriate deuterated internal standards were employed in each case. Quantification limits were in the range of 0.8–15 ng/l and 0.1–46 ng/g dw, whereas detection limits achieved ranged from 0.3 to 5 ng/l and from 0.03 to 13 ng/g dw in waters and sediments, respectively. The accuracy of the method ranged

from 42 to 117% (Table S3 in supplementary material). Precision of the method expressed as repeatability and reproducibility was below 11% and 18% respectively, in all cases (Table S3 in supplementary material).

2.6.4.2. Personal care products (PCPs). Eight musks (galaxolide, galaxolidone, tonalide, celestolide, phantolide, traseolide, musk xylene and musk ketone) and five UV filters (homosalate, oxybenzone, EHMC, 4MBC and octocrylene) were analysed in water, sediment and oyster samples. Water samples extraction (by SPE), sediment extraction (by ultrasound assisted solvent extraction) and oyster tissue extraction (by salting-out liquid-liquid extraction and clean-up by dispersive solid-phase extraction) and respective quantifications were carried out as described above for PAHs.

Tonalide d_3 and octocrylene d_{15} were used to quantify the structurally related musks and UV filters, with recoveries in the acceptability range of 80–120% and reproducibility between 3 and 47% than (Table S4 in supplementary material). Limits of detection and limits of quantification ranged from 0.1 to 10 and from 0.5 to 32 ng/g dw, respectively, for sediment and oysters. For water samples, limits of detection and quantification varied between 0.07 and 7.2 ng/l and 0.2 and 24 ng/l, respectively.

2.6.5. Pesticides

Twenty six pesticides were assessed in water and sediment samples. No analyses in oyster tissue were carried out as no analytical methodology was available. However, determination of the presence of these contaminants in the environmental can contribute for assessment of a potential exposure risk of oysters.

Pesticides thiamethoxam, dimethoate, acetamiprid, thiacloprid, fluometuron, chlorantraniliprole, metalaxyl, diuron, boscalid, myclobutanil, linuron, s-metolachlor, prometryn, tebupirimfos, chlorpyrifos, fluazifop-p-butyl, imazalil, fenpyroximate, irgarol and azamethiphos (group I) were analysed by LC–LTQ/Orbitrap MS, after being extracted by means of SPE from water and a modified QuEChERS approach from sediments (Nannou et al., 2018).

Pesticides ethoxyquine, chlorothalonil, chlorpyriphos-methyl, chlorpyriphos, resmethrin and λ-cyhalothrin (group II) were extracted from water according to Hladik et al. (2009) and from sediments according to González-Curbelo et al. (2015), using SPE and GC–MS for analysis.

In oyster tissues, only the pesticides azamethiphos and irgarol were measured, using a QuEChERS-based modified method, adapted from Rawn et al. (2010), followed by LC–LTQ/Orbitrap MS analysis.

All results were confirmed by MS2 fragmentation. Details are presented in Supplementary material S1.

Recoveries between 51 and 111% for water samples, between 50 and 112% for sediments and between 69 and 74% for oysters were obtained (Table S5 in supplementary material). Repeatability was below 12% (Table S5 in supplementary material). Limits of detection and quantification ranged from 0.5 to 4 and from 2.0 to 13 ng/g lw, respectively, for oysters' tissues and from 0.1 to 9 and from 0.5 to 30 ng/g dw, respectively, for sediments. For waters, limits of detection and limits of quantification ranged from 0.1 to 30 and from 0.3 to 90 ng/l.

2.6.5.1. Pyrethroid insecticides. Nine pyrethroid insecticides, including, bifenthrin, cyfluthrin, cyhalothrin, cypermethrin, deltamethrin, fenvalerate, fluvalinate, permethrin and tetramethrin were analysed in water, sediment and oyster samples. In oyster samples, the insecticides allethrin, flumethrin, kadethrin, prealethrin and transfluthrin were also measured.

Extraction of pyrethroids from water was carried out by ultrasound-assisted emulsification-extraction (Feo et al., 2010a). In sediments and oysters' tissues, pyrethroids were extracted in an ultrasonic bath and extracts were cleaned up by SPE using Florisil cartridges for sediments, and a C18 cartridge in tandem with a basic alumina cartridge for oysters (Feo et al., 2010b; Feo et al., 2011). Analysis was performed by GC–MS in negative chemical ionisation (NCI) mode with ammonia as the reagent gas (Feo et al., 2011). Details are presented in Supplementary material S1.

Recovery percentages between 55 and 106% were obtained, being always within the range of acceptability (40–120%) for analytical methods based on quantification by isotopic dilution (Table S6 in supplementary material). Reproducibility percentages between 1.3 and 9.3% were also achieved. Limits of detection and limits of quantification ranged from 0.004 to 6.4 and from 0.01 to 21 ng/g lw, respectively for oysters. For sediments, between 0.0001 and 0.01 and 0.0003 and 0.05 ng/g dw. For water samples, between 0.07 and 5 and 0.2 and 7 ng/l.

2.6.6. Other compounds

Preservatives, anti-bacterial, insect repellent and anti-fouling compounds (methyl paraben, propyl paraben, triclosan, DEET and seanine) were analysed in water, sediments and oyster samples using the methodology described for PAHs analysis. Recovery and limits of detection and quantification were in the same ranges as those reported for PAHs (Tables S7 and S8 in supplementary material).

2.7. Statistical analysis

Three independent replicates were carried out per season to determine each group of parameters. Data were analysed statistically using analysis of variance (ANOVA) and the Tukey pair wise comparison was employed to determine the significance of the differences among samples from the four season sites at a confidence limit of 95%. The statistical package used was GraphPad Prism 6 software. Multivariate analysis including correlations and principal components analysis (PCA) among data was carried out using Primer 6 software package.

3. Results

3.1. Oyster morphological, quality and nutritional parameters

All collected oysters were within commercial size, with length between 9 and 11 cm (Table 1). Significant differences ($P < 0.05$) were observed among seasons: total body weight was higher in winter and lower in spring, however the edible portion was heavier in oysters collected in summer.

Regarding the quality and nutritional parameters, significant differences ($P < 0.05$) were observed among seasons. Higher values of protein, lipids and energy and lower glycogen and moisture content were observed in summer (S1), while the opposite pattern occurred in autumn (S2) and winter (S3) (Table 2). The AFNOR quality index was significantly higher in summer (S1) when oysters achieved their highest edible portion content, compared to the other seasons (Table 1), Overall, AFNOR index was considered very high during the four sampling periods.

Table 1
Biometrical measurements (average length, average total weight and average fresh weight of tissue per individual, n = 20) of farmed oysters collected in Canal de Mira, Aveiro district on each season. S1 – Summer, July 2016, S2 – Autumn, November 2016, S3 – Winter, January 2017, S4 - Spring, May 2017.

	S1	S2	S3	S4
Length (cm)	9.0 ± 0.8	9 ± 1	11 ± 2	9 ± 1
Total weight (g)	74 ± 5^{b}	78 ± 2^{b}	86 ± 5^{a}	59 ± 2^{c}
Tissue weight (g)*	$22 \pm 5^{a,b}$	26 ± 5^{a}	26 ± 6^{a}	16 ± 3^{b}
Edible portion (g)	16.5 ± 0.3^{a}	14.2 ± 0.2^{b}	14.0 ± 0.3^{b}	9.9 ± 0.4^{c}
AFNOR quality index	23 ± 1^{a}	18.4 ± 0.6^{b}	16.6 ± 0.8^{b}	16.9 ± 0.9^{b}

Mean values within each row not sharing a common superscript were significantly different (ANOVA ($P^{-}0.05$)), *with intervalvar liquid.

Table 2
Seasonal variation quality and nutritional parameters (mean values ± standard deviation, n = 5 of pooled samples/season) of farmed oyster in Canal de Mira, Aveiro district. S1 – Summer, July 2016, S2 – Autumn, November 2016, S3 – Winter, January 2017, S4 - Spring, May 2017.

	S1	S2	S3	S4
Moisture (%)	76.3 ± 0.8 [b]	79 ± 1 [a]	80.6 ± 0.8 [a]	79.1 ± 0.3 [a]
Ash (%ww)	2.4 ± 0.2 [b]	2.3 ± 0.1 [b]	2.4 ± 0.2 [b]	2.7 ± 0.2 [a]
Crude protein (%ww)	15.1 ± 0.4 [a]	11.1 ± 0.5 [c]	10.3 ± 0.4 [c]	12.7 ± 0.5 [b]
Crude lipids (%ww)	3.5 ± 0.8 [a]	2.6 ± 0.3 [ab]	2.4 ± 0.2 [b]	2.6 ± 0.2 [ab]
Total glycogen (%ww)	1.7 ± 0.3 [b]	5.29 ± 0.9 [a]	5.1 ± 0.5 [a]	2.4 ± 0.3 [b]
Gross energy (kJ/g)	5.2 ± 0.4 [a]	4.4 ± 0.3 [b]	4.0 ± 0.2 [b]	4.35 ± 0.09 [b]

Mean values within each row not sharing a common superscript were significantly different (ANOVA (P˂0.05)).

3.2. Water physical-chemical parameters

The results of the seasonal physical-chemical analysis of water are summarized in Table S9 in supplementary material. Water temperature ranged between 18.4 °C in summer (S1) and 10.8 °C in winter (S3). The pH presented small fluctuations among seasons, averaging 7.6. Although with a peak in summer (S1), turbidity values were low, ranging from 0.6 to 2.1 NTU. As expected, dissolved oxygen decreased in spring and summer months, contrarily to the salinity and Chl*a* levels. Small fluctuations in PM content could be observed, and a modest increase could be detected in spring, essentially due to the increase of POM.

Dissolved carbon was identical among seasons, whereas nitrogen (in its different forms) showed higher levels in colder seasons, i.e. autumn (S2) and winter (S3). The same trend was observed for phosphorus and ammonium concentrations.

3.3. Sediment physical-chemical characteristics

Sediment samples from autumn (November 2016) were not collected due to logistic problems. Organic matter content in sediments ranged between 1.82 and 4.26%, showing lower values in spring (S4). The grain size analysis revealed that sediments from oyster's bed were constituted mainly (>90%) by small size particles (<1 mm) and its composition was rather stable among seasons (Table S10 in supplementary material).

3.4. Chemical contaminants

3.4.1. Water samples

In general, no PAHs were detected. The only exception was acenaphthylene that was detected at all seasons (with no significant differences registered among seasons) and anthracene in autumn (S2) (Table 3, Fig. 2).

Butyltins were all below the detection limits.

Regarding OPFRs, only TCPP and EHDPP were not detected in the samples (Table 4), whereas the remaining five OPFRs (TiBP, TNBP, TCEP, TDCPP and TPHP) were detected at least in one of the collected water samples. In general, the highest values were detected in autumn (S2), whereas the lowest values were found in spring (S4).

For PPCPs, out of the twenty-five pharmaceuticals measured in water samples, only paracetamol (12 ng/l), atenolol (below quantification limit, <3 ng/l) and citalopram (below quantification limit, <2 ng/l) were detected in water, but only in samples collected in the summer (S1). In the remaining seasons all pharmaceuticals were below the detection limits (Table S3 in supplementary material). For musks, out of the eight assessed, only galaxolidone was detected in water, presenting higher concentration in autumn (S2) and lower in spring (S4) (Table 6). Regarding UV filters, only homosalate was not detected (Table 6). The remaining four compounds were detected in at least two out of the four samples, with their concentrations being higher either in summer (S1, octocrylene and EHMC) or in autumn (S2, oxybenzone and 4MBC) and lower in spring (S4).

Out of the twenty six pesticides measured in water only three were detected, but the concentrations were below the quantification limits, being detected in only one season (metalaxyl and irgarol in summer (S1)) or two (prometryn in winter (S3) and spring (S4)). Regarding the nine pyrethroids insecticides measured, only cypermethrin was detected (31 ng/l) in water samples collected in winter (S3). Limits of detection are shown in Tables S5 and S6 in supplementary material.

Among the preservatives, anti-bacterial, insect repellent and anti-fouling compounds, only DEET was not detected (Table S8 in supplementary material). The remaining compounds were detected in almost all samples, but no clear seasonal profile was observed. Nonetheless, all remaining compounds were detected in summer (S1).

3.4.2. Sediments samples

With the exception of naphthalene and acenaphthene, which were not detected in any sample, all other fourteen PAHs were detected in at least one sediment sample (Table 3). In general, individual PAHs

Table 3
Polycyclic aromatic hydrocarbons (PAHs) concentrations (mean values ± standard deviation, n = 3) determined in water, sediment and oyster tissues samples collected at oyster aquacultures farm in Canal de Mira, Aveiro district. S1 – Summer, July 2016, S2 – Autumn, November 2016, S3 – Winter, January 2017, S4 - Spring, May 2017.

Polycyclic aromatic hydrocarbons	Water (ng/L)				Sediment (ng/g dw)				Oyster (ng/g dw)			
	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
Naphthalene	<15*	<15*	<15*	<15*	<3.6*	<3.6*	<3.6*	<3.6*	NA	NA	NA	NA
Acenaphthylene	7 ± 5	18 ± 21	2.7 ± 0.9	4 ± 3	0.5 ± 0.1[a]	1.2 ± 0.2[b]	11 ± 7[c]	3 ± 3[b,c]	<2.9*	<2.9*	<2.9*	<2.9*
Acenaphthene	<1.1*	<1.1*	<1.1*	<1.1*	<0.21*	<0.21*	<0.21*	<0.21*	<4.7*	<4.7*	<4.7*	<4.7*
Fluorene	<0.69*	<0.59*	<0.59*	<0.59*	1.2 ± 0.7	<0.31*	<0.31*	1.5 ± 0.6	12 ± 7	24 ± 2	21 ± 11	<2.3*
Phenanthrene	<4.0*	<4.0*	<4.0*	<4.0*	6.5 ± 0.6	5 ± 4	<4.8**	5 ± 2	<5.7**	11 ± 5[a]	50 ± 9[b]	<1.70*
Anthracene	<0.08*	3 ± 1	<0.08*	<0.08*	<3.9**	<1.2*	<3.9**	<3.9**	<2.4*	13 ± 6	11 ± 7	<2.4*
Fluoranthene	<0.68*	<0.68*	<2.3**	<0.68*	6 ± 2	17 ± 16	4 ± 2	4 ± 2	<2.9*	14 ± 3[a]	59 ± 9[b]	<9.6**
Pyrene	<0.56*	<0.56*	<0.56*	<0.56*	5 ± 1	11 ± 10	4 ± 2	4 ± 1	<11**	26 ± 1[a]	57 ± 10[b]	30 ± 9[a]
Benz(a)anthracene	<0.08*	<0.08*	<0.08*	<0.08*	4 ± 1	10 ± 9	<2.0**	3 ± 1	<2.8*	<2.8*	<2.8*	<2.8*
Chrysene	<0.02*	<0.02*	<0.02*	<0.02*	2 ± 1	7 ± 6	1.6 ± 0.7	2 ± 1	<2.4*	<2.4*	<2.4*	<2.4*
Benzo(b)fluoranthene	<0.04*	<0.04*	<0.04*	<0.04*	5 ± 1	13 ± 11	3 ± 1	2 ± 2	<1.9*	<1.9*	<1.9*	<1.9*
Benzo(k)fluoranthene	<0.06*	<0.06*	<0.06*	<0.06*	2 ± 0.6	3 ± 2	1.1 ± 0.1	1.4 ± 0.6	<1.9*	<1.9*	<1.9*	<1.9*
Benzo(a)pyrene	<0.03*	<0.03*	<0.03*	<0.03*	2.5 ± 0.8	7 ± 6	2 ± 1	3 ± 1	<3.5*	<3.5*	<3.5*	<3.5*
Indenopyrene	<0.16*	<0.16*	<0.16*	<0.16*	4 ± 2	7 ± 5	3.8 ± 0.1	5 ± 3	3.9 ± 0.7[a,b]	<2.8**	2.8 ± 0.7[a]	5 ± 1[b]
Dibenz(a,h)anthracene	<0.21*	<0.21*	<0.21*	<0.21*	<1.4**	<1.4**	<0.40*	<1.4**	<1.6*	<1.6*	<1.6*	<1.6*
Benzo(ghi)perylene	<0.29*	<0.29*	<0.29*	<0.29*	3 ± 2	6 ± 5	2 ± 1	3 ± 1	<1.9*	<1.9*	<1.9*	<1.9*
∑PAHs	7	20	2.7	4	46	87	32	38	16	88	200	34

* Limit of detection; ** limit of quantification; NA: not analysed; dw: dry weight, mean values within each row not sharing a common superscript (a,b,c…) were significantly different (ANOVA (P˂0.05)).

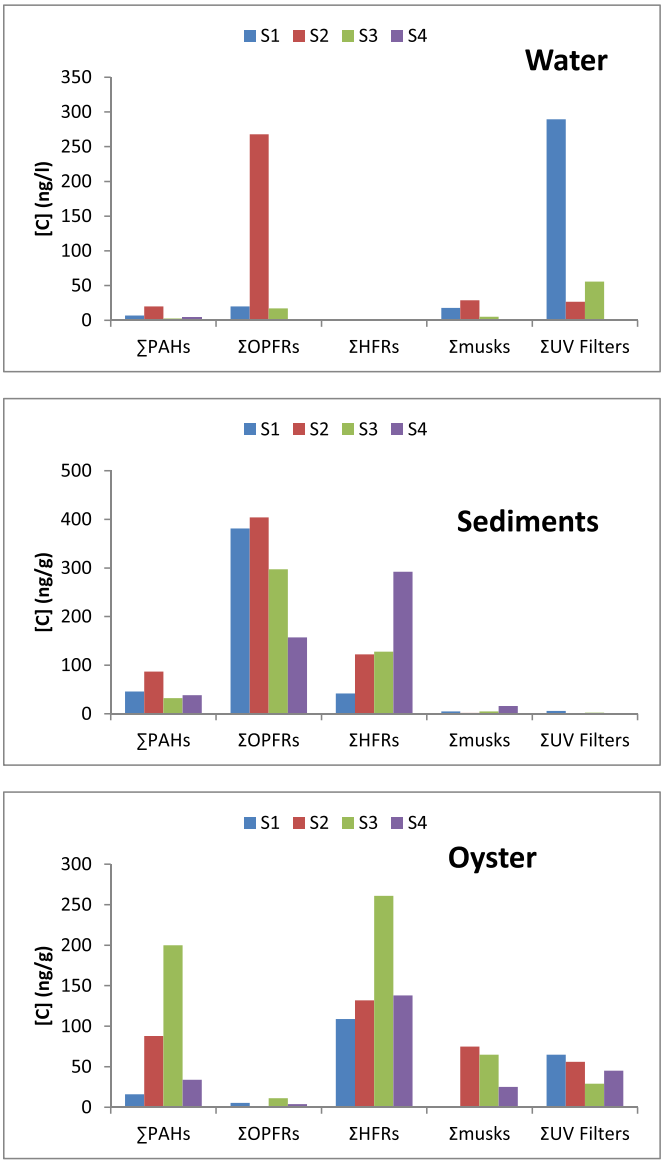

Fig. 2. Total concentration of the different families of organic contaminants detected in water, sediment and oysters tissues. S1 – Summer, July 2016, S2 – Autumn, November 2016, S3 – Winter, January 2017, S4 - Spring, May 2017.

values were low, ranging from 0.5 and 17 ng/g dw. Seasonal variations were only significant for acenaphthylene, which presented the lowest values in summer (S1) and the highest in winter (S3). Nonetheless, total PAHs concentration (sum of concentrations of all PAHs quantified) was higher in autumn (S2) (Fig. 2).

Butyltins were below the detection limits in all sediment samples.

Several of the 16 OPFRs were detected in at least one sediment sample (Table 4), with only five not detected in any samples (TiBP, TNBP, TBP, IDPP and THP). IPPP was the one with the highest concentration in summer and autumn, whereas EHDPP was the one detected in higher amounts in winter and spring. In general, the lowest values were observed in spring (S4), whereas the highest values were found either in summer (S1) or autumn (S2), depending on the OPFR. However, the sum of all OPFRs analysed was higher in autumn (S2) (Fig. 2).

Regarding HFRs, out of the thirteen analysed, only BDE-100, BDE-209 and Dec 602 were detected (Table 5). BDE-100 was only detected in summer (S1), whereas BDE-209 and Dec 602 were detected in all samples, being higher in spring (S4) samples, which also showed the highest total HFRs amount (Fig. 2).

Out of the twenty-five pharmaceuticals measured, only budesonide was detected (below quantification limit, <0.1 ng/g dw), and only in sediment samples collected in the summer (S1). In the remaining samples all pharmaceuticals were below the detection limits (Table S3 in supplementary material). The only musk detected in sediment samples was tonalide, with lower levels registered in autumn (S2) (Table 6). Out of the five UV filters, only EHMC (in summer, S1) and octocrylene (in summer and winter, S1 and S3) were detected (Table 6).

None of the thirty five pesticides (including the nine pyrethroids insecticides) analysed were detected in sediment samples (limits of detection included in Tables S5 and S6 in supplementary material).

Among the preservatives, anti-bacterial, insect repellent and anti-fouling compounds, methyl paraben, propyl paraben and triclosan were detected in two sediment samples with no clear seasonal pattern (Table S8 in supplementary material).

3.4.3. Oyster samples

Out of the 16 PAHs analysed, only 6 were detected in at least one oyster tissue sample (Table 3). The remaining PAHs were not detected in any sample. Among the PAHs detected, five were among the lighter ones (fluorene, phenanthrene, anthracene, fluoranthene and pyrene), whereas only one of the heaviest PAHs, indenopyrene, was detected. In general, the lighter PAHs were present in higher amounts in cooler seasons, i.e. autumn and winter (S2 and S3, respectively), whereas for indenopyrene the opposite trend was observed. A significant negative correlation with water temperature was observed (Table S11 in supplementary material). In general, PAHs levels were low with individual PAHs concentrations ranging from 3 to 60 ng/g (dw). Total PAHs ranged from 16 to 200 ng/g (dw), with the highest amount being observed in winter (S3) (Fig. 2). In addition, seasonal correlations between total PAHs and oysters nutritional composition were observed (Fig. S1 in supplementary material).

Butyltins were below the detection limits.

Out of the sixteen OPFRs analysed only TPPO, TDCPP and DCP were detected in one or two oyster's tissues samples, with no clear seasonal pattern registered and at very low concentration levels (Table 4) and no correlation with water temperature (Table S11 in supplementary material).

Out of the thirteen HFRs determined, only BDE-47, BDE-209 and Dec 602 were detected (Table 5). The two PBDEs were detected in all samples, being BDE-209 and Dec 602 higher in winter oysters' tissues (S3), whereas PBDE-47 was higher in summer (S1) but no correlation with water temperature was observed (Table S11 in supplementary material).

Within musks, galaxolide and galaxolidone were the only ones detected (out of the eight determined), showing higher values in autumn (S2) and winter (S3) (Table 6). On the other hand, homosalate and octocrylene (the only UVs filters detected among the five analysed) were lower in winter (S3) (Table 6), being positively correlated with water temperature (Table S11 in supplementary material).

The two pesticides analysed were not detected in oyster's tissues (limits of detection provided in Table S5 in supplementary material). Regarding pyrethroid insecticides, out of the fourteen compounds analysed, only five were detected in one or two samples: tetramethrin (below quantification limit (<1 ng/g lw), in autumn (S2)), bifenthrin (0.03 ng/g lw in summer (S1)), cyhalothrin (0.07 ng/g lw, in summer (S1)), fenvalerate (below quantification limit (<0.2 ng/g lw), in summer (S1) and autumn (S2)) and permethrin (below quantification limit (<0.2 ng/g lw) in autumn (S2) and winter (S3) and 1.1 ng/g lw in summer).

None of the preservatives, anti-bacterial, insect repellent and anti-fouling were detected in oyster's tissues (Table S8 in supplementary material).

Table 4
Organophosphate flame retardants (OPFRs) (mean values ± standard deviation, n = 3) determined in water, sediment and oyster tissues samples collected at oyster aquacultures farm in Canal de Mira, Aveiro district. S1 – Summer, July 2016, S2 – Autumn, November 2016, S3 – Winter, January 2017, S4 - Spring, May 2017.

OPFRs	Water (ng/L)				Sediment (ng/g dw)				Oyster (ng/g lw)			
	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
TiBP	<15*	98 ± 45	<15*	<15*	<0.99*	<0.99*	<0.99*	<0.99*	<12*	<12*	<12*	<12*
TnBP	6 ± 1[a]	46 ± 14[b]	7 ± 2[a]	<0.8*	<0.32*	<0.32*	<0.32*	<0.32*	<6.5*	<6.5*	<6.5*	<6.5*
TCEP	9.8 ± 0.9[a]	88 ± 25[b]	<0.13*	<0.13*	<0.07*	2.8 ± 0.1	<0.07*	<0.07*	<3.5**	<1.2*	<1.2*	<1.2*
TPPO	NA	NA	NA	NA	35 ± 1	<0.85*	<2.5**	<0.85*	5.4 ± 0.1	<0.35*	<1.3**	<1.3**
TClPP	<175*	<175*	<175*	<175*	115 ± 3[a]	<0.09*	94 ± 3[b]	7.4 ± 0.2[c]	<1.5*	<1.5*	<1.5*	<1.5*
TDCPP	3.7 ± 0.4[a]	21 ± 2[b]	10 ± 3[c]	<0.15*	0.35 ± 0.03	0.46 ± 0.04	<0.05*	<0.05*	<1.0**	<0.19*	2.6 ± 0.2[a]	3.7 ± 0.3[a]
TPHP	<3.9*	15 ± 2	<3.9*	<3.9*	1.33 ± 0.02	<0.08*	1.50 ± 0.02	<0.08*	<3.4**	<1.3*	<1.3*	<1.3*
TBP	NA	NA	NA	NA	<0.03*	<0.03*	<0.03*	<0.03*	<3.4*	<3.4*	<3.4*	<3.4*
DCP	NA	NA	NA	NA	2.1 ± 0.2	<0.06*	2.2 ± 0.2	<0.06*	<1.6*	<1.6*	8.6 ± 0.3	<1.6*
TBOEP	NA	NA	NA	NA	10.4 ± 0.2[a]	0.220 ± 0.003[b]	1.83 ± 0.03[c]	2.10 ± 0.03[c]	<1.4**	<0.44*	<0.44*	<0.44*
TMCP	NA	NA	NA	NA	<0.09*	24 ± 1[a]	1.38 ± 0.07[b]	<0.09*	<2.5*	<2.5*	<2.5*	<2.5*
EHDP	<19*	<19*	<19*	<19*	<0.02*	98 ± 1[a]	183 ± 2[b]	92 ± 1[a]	<0.53*	<0.53*	<0.53*	<0.53*
IDPP	NA	NA	NA	NA	<0.05*	<0.05*	<0.05*	<0.05*	<3.0*	<3.0*	<3.0*	<3.0*
IPPP	NA	NA	NA	NA	175 ± 4[a]	279 ± 6[b]	<1.2*	47 ± 1[c]	<25**	<19*	<19*	<19*
THP	NA	NA	NA	NA	<0.06*	<0.06*	<0.06*	<0.06*	<0.88*	<0.88*	<0.88*	<0.88*
TEHP	NA	NA	NA	NA	42 ± 2[a]	<0.1*	11.4 ± 0.6[b]	8.5 ± 0.5[b]	<1.9*	<1.9*	<1.9*	<1.9*
ΣOPFRs	20	268	17	–	381	404	297	157	5.4	–	11	3.7

* Limit of detection; ** limit of quantification; NA: not analysed; dw: dry weight; lw: lipidic weight, mean values within each row not sharing a common superscript (a,b,c…) were significantly different (ANOVA (P˙0.05)).

4. Discussion

Oysters' nutritional profile was within the range of values previously reported for *C. gigas* (Dridi et al., 2007; Linehan et al., 1999), showing that it is a rich source of protein (10–15% WW) with low fat content (≤3.5% WW) all year round. The biochemical composition and quality of oysters showed a clear seasonal variation. Seasonal changes in the biochemical composition of bivalves' edible portion can result from interactions among environmental conditions (mainly food availability), growth and the reproductive cycle (Dridi et al., 2007; Ojea et al., 2004; Rodríguez-Jaramillo et al., 2008). A study in *C. gigas* showed an inverse relationship between glycogen and lipid levels, glycogen decreased prior to spawning while lipid accumulated during gonad ripeness (Dridi et al., 2007), which suggests that during our summer sampling, oysters were probably fully mature and therefore, heavier and nutritionally richer. Oysters showed a premium quality all year round with an AFNOR quality index higher than 16.6, well above the limit of 9 established for high quality oysters (Goyard, 1995). Oysters' quality and nutritional parameters are strongly dependent on nutrients, organic matter and microalgae availability. For instance, turbidity and concentration of phytoplankton were higher in summer, thus affecting feed consumption and oysters' physiology. Several environmental factors can also interfere with oysters' filtration function, such as temperature, salinity and suspended particulate concentration, which were generally higher in summer. Overall, despite the seasonal variations on the biochemical composition, the oysters cultured in Ria de Aveiro presented an elevated quality over the four sampling periods, which highlights the great potential of this production area.

Although the levels of pollutants in the environment likely affect their presence and concentration in organisms living/growing in that environment, their accumulation depends on the pollutant and organism. In the present study, the distribution of pollutants among the three matrices was not always directly linked.

Most pollutants were below the detection limits in water, sediment and oysters, but some of them were detected in the three matrices analysed. This was the case of PAHs. PAHs are ubiquitous in the environment having different sources, such as incomplete combustion of fossil fuel (producing mainly high molecular weight PAHs), and from accidental oil spillages/discharges (producing mainly low molecular weight PAHs) (Baumard et al., 1998). Because of their hydrophobicity (log Kow > 4), PAHs are usually not detected in water samples but in sediments, particularly thin sediments with high organic matter content

Table 5
Halogenated flame retardants (HFRs), including polybrominated diphenyl ethers (PBDEs) congeners and dechloranes (Dec) (mean values ± standard deviation, n = 3) determined in sediment and oyster tissues samples collected at oyster aquacultures farm in Canal de Mira, Aveiro district. S1 – Summer, July 2016, S2 – Autumn, November 2016, S3 – Winter, January 2017, S4 - Spring, May 2017.

HFRs	Sediment (ng/g dw)				Oyster (ng/g lw)			
	S1	S2	S3	S4	S1	S2	S3	S4
BDE-28	<0.01*	<0.01*	<0.01*	<0.01*	<0.04*	<0.04*	<0.04*	<0.04*
BDE-47	<0.01*	<0.01*	<0.01*	<0.01*	0.308 ± 0.004	<0.18**	0.18	0.19
BDE-99	<0.03*	<0.03*	<0.03*	<0.03*	<0.3*	<0.3*	<0.3*	<0.3*
BDE-100	0.79 ± 0.04	<0.02*	<0.02*	<0.02*	<0.2*	<0.2*	<0.2*	<0.2*
BDE-154	<0.08*	<0.08*	<0.08*	<0.08*	<0.4*	<0.4*	<0.4*	<0.4*
BDE-153	<0.1*	<0.1*	<0.1*	<0.1*	<0.6*	<0.6*	<0.6*	<0.6*
BDE-183	<0.4*	<0.4*	<0.4*	<0.39*	<3.2*	<3.2*	<3.2*	<3.2*
BDE-209	14.3 ± 0.9[a]	54 ± 3[b]	<5.5**	75 ± 4[c]	75 ± 5[a]	132 ± 8[b]	160 ± 10[c]	137 ± 8[b]
ΣPBDEs	15	54	–	75	76	132	160	138
Dec 602	26 ± 1[a]	67 ± 3[b]	127 ± 5[c]	218 ± 9[d]	34 ± 4[a]	<0.02*	101 ± 12[b]	<0.02*
Dec 603	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.007*	<0.007*	<0.007*	<0.007*
Dec 604	<0.0002*	<0.0002*	<0.0002*	<0.0002*	<0.007*	<0.007*	<0.007*	<0.007*
syn-DP	<0.0003*	<0.0003*	<0.0003*	<0.0003*	<0.005*	<0.005*	<0.005*	<0.005*
anti-DP	<0.0002*	<0.0002*	<0.0002*	<0.0002*	<0.002*	<0.002*	<0.002*	<0.002*
ΣHFRs	42	122	128	292	109	132	261	138

* Limit of detection; ** limit of quantification; NA: not analysed; dw: dry weight; lw: lipidic weight, mean values within each row not sharing a common superscript (a,b,c…) were significantly different (ANOVA (P˙0.05))

Table 6
Personal care products (PCPs), including musks and UV filters (mean values ± standard deviation, n = 3) determined in water, sediment and oyster tissues samples collected at oyster aquacultures farm in Canal de Mira, Aveiro district. S1 – Summer, July 2016, S2 – Autumn, November 2016, S3 – Winter, January 2017, S4 - Spring, May 2017.

Musks	Water (ng/L)				Sediment (ng/g dw)				Oyster (ng/g dw)			
	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
Galaxolide	<2.7*	<2.7*	<2.7*	<2.7*	<1.2*	<1.2*	<1.2*	<1.2*	<22**	61 ± 3[a]	58 ± 7[a]	25 ± 1[b]
Galaxolidone	18 ± 1[a]	29 ± 1[b]	5 ± 1[c]	<1.4*	<0.26*	<0.26*	<0.26*	<0.26*	<2.1*	14 ± 5	7 ± 3	<2.1*
Tonalide	<1.2*	<1.2*	<1.2*	<1.2*	5 ± 1[a]	1.8 ± 0.5[b]	5 ± 2[a]	16 ± 11[a,b]	NA	NA	NA	NA
Celestolide	<0.07*	<0.07*	<0.07*	<0.07*	<0.21*	<0.21*	<0.21*	<0.21*	<3.0*	<3.0*	<3.0*	<3.0*
Phantolide	<0.06*	<0.06*	<0.06*	<0.06*	<0.15*	<0.15*	<0.15*	<0.15*	<6.5*	<6.5*	<6.5*	<6.5*
Traseolide	<0.11*	<0.11*	<0.11*	<0.11*	<0.27*	<0.27*	<0.27*	<0.27*	<1.8*	<1.8*	<1.8*	<1.8*
Musk xylene	<0.88*	<0.88*	<0.88*	<0.88*	<0.23*	<0.23*	<0.23*	<0.23*	<3.2*	<3.2*	<3.2*	<3.2*
Musk ketone	<0.13*	<0.13*	<0.13*	<0.13*	<0.43*	<0.43*	<0.43*	<0.43*	<3.1*	<3.1*	<3.1*	<3.1*
Σmusks	18	29	5	–	5	1.8	5	16	–	75	65	25
UV filters												
Homosalate	<1.9*	<1.9*	<1.9*	<1.9*	<0.82*	<0.82*	<0.82*	<0.82*	45 ± 7[a]	35 ± 4[a]	20 ± 3[b]	29 ± 3[a,b]
Oxybenzone	<6*	24 ± 6	<20**	<6*	<1.7*	<1.7*	<1.7*	<1.7*	<9.5*	<9.5*	<9.5*	<9.5*
EHMC	8.5 ± 0.4[a]	<0.42*	1.7 ± 0.7[b]	<0.42*	1.1 ± 0.2	<0.33*	<0.33*	<0.33*	<1.8*	<1.8*	<1.8*	<1.8*
4MBC	<0.99**	2.6 ± 0.6	<0.33*	<0.33*	<0.61*	<0.61*	<0.61*	<0.61*	<6.0*	<6.0*	<6.0*	<6.0*
Octocrylene	281 ± 19[a]	<7.2*	54 ± 22[b]	<7.2*	5 ± 1[a]	<0.64*	2.5 ± 0.2[b]	<0.64*	20 ± 3[a]	21 ± 3[a]	9 ± 3[b]	16 ± 5[a,b]
ΣUV Filters	290	27	56	–	6	–	3	–	65	56	29	45

* Limit of detection; ** limit of quantification; NA: not analysed; dw: dry weight, mean values within each row not sharing a common superscript (a,b,c…) were significantly different (ANOVA (P<0.05)).

as those in this study. In fact, in the present study only acenaphthylene, one of the lighter and less hydrophobic PAHs, was detected in water, whereas almost all analysed PAHs were detected in sediments. Nevertheless, total PAHs sediment levels were lower than those reported for instance in Douro estuary in NW Portugal and in other highly urbanized coastal areas (Rocha et al., 2011). PAHs are also readily absorbed and rapidly distributed within the organisms body (especially those with high fat content) because they are highly lipid soluble (Abdel-Shafy and Mansour, 2016). Seasonal PAHs accumulation was also observed, with levels in oyster tissues increasing from spring to winter and decreasing in summer. The PAH concentration range was larger in tissues (∑PAHs 16–200 ng/g dw) than in sediment (∑PAHs 32–87 ng/g dw), even though both compartments are considered integrative. Loh et al. (2018) reported that interactions between PAHs and suspended particulate matter could affect PAHs bioaccumulation, lowering PAHs availability. This corroborated the obtained results, as turbidity and particulate matter were low in water during winter, one can assume higher availability in winter. On the other hand, in winter oysters were bigger. Bigger animals can have increased filtration processes that can result in higher pollutant accumulation and seasonal correlations were observed between total PAHs concentrations and oysters nutritional composition. With lower temperatures and less food, their growth rate is also lower in winter, causing less growth dilution in the tissues in comparison with spring and summer. Nevertheless, the higher PAHs levels in water and sediment were observed in autumn, indicating that a direct relationship between environmental PAHs concentrations and oysters' bioaccumulation is limited by the oyster life cycle. PAHs raise great concern when present in food items, due to their known toxic, mutagenic and carcinogenic effects. In 2011 (ECR, 2011b) the maximum residue levels allowed for PAHs in fish, crustaceans and molluscs were reduced, and in foodstuffs one must now screen not only benzo(a)pyrene, but also benzo(a)anthracene, benzo(a)fluoranthene and chrysene. None of these PAHs were detected in oyster tissues in the current study. On the other hand, EFSA CONTAM Panel concluded that eight PAHs (benzo[*a*]pyrene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, chrysene, dibenz[*a,h*]anthracene and indeno[1,2,3-*cd*]pyrene), either individually or combined, are currently the only indicators of the carcinogenic potential of PAHs in food, establishing toxicological guidance values (EFSA, 2008c). Of these only indeno[1,2,3-*cd*]pyrene was detected, but in low levels (ca. 4 ng/g dw), indicating that no toxicological risks are expected.

Regarding BTs, none was detected either in water, sediment or oyster tissues. TBT is a biocide commonly used in antifouling paints of ship hulls, which was banned worldwide in 2008 (Price and Readman, 2013). TBT and its degradation products, MBT and DBT, are persistent in the marine environment. Their non-detection might reflect almost ten years of non-usage of TBT products. New antifouling products, such as seanine, have been replacing these old ones. Seanine belongs to a new generation of environmentally acceptable marine antifouling paints for ships and marine structures. In the present study, this compound was detected in water in summer, a season of higher touristic activity with increased recreational boats circulating at the studied area, suggesting a need to regularly monitor this compound in water. Nonetheless, seanine, of known low persistence (Thomas et al., 2002), was not detected either in sediment or in oyster tissues.

The Canal de Mira aquaculture facility is located in an area subject to some anthropogenic activity due to touristic activities and in the summer it is subjected to a huge increase of tourists. The population increase can also result in higher volumes of wastewater effluent discharges, which can affect the aquaculture production. In fact, this touristic activity is reflected in the detection of several pollutants in higher amounts in summer. For instance, six PCPs (one musk, galaxolidone, two UV-filters, EHMC and octocrylene, two preservatives, methyl paraben and propyl paraben and the antimicrobial triclosan) were found in water at higher levels in summer. Tonalide and galaxolide, the precursor of galaxolidone, are generally the musks most frequently detected in water (Smyth et al., 2008; Rosal et al., 2010), but not in the present study. Tonalide was however detected in sediments. EHMC and octocrylene were also detected in sediments, mainly in summer. This unique group of compounds, along with pharmaceutical compounds (which were not detected in the present study), are used to improve our daily life quality, but are of emerging environmental concern. Pharmaceuticals are designed to be biologically active and can pose a risk for aquatic organisms even at low concentrations (Gago-Ferrero et al., 2013; Ebele et al., 2017). Some of these compounds exhibit endocrine disrupting properties and in many regions (e.g., European Union (EU), United States of America (USA), Japan and Australia) UV-filters and musks have strict legislation in terms of their manufacture and utilization in product formulation (Cunha et al., 2018). These compounds are released into the aquatic environment mostly through wastewater treatment plants (WWTPs) effluents. Population peak in summer season can lead to an increase of pharmaceuticals and PCPs in water. However, due to their lipophilic nature (log Kow values between 4 and 8) and stability in the environment, compounds such as UV filters can bioaccumulate. In fact, a few studies reported that both musks and UV-filters have been detected in various aquatic organisms, including fish, mussels, clams and dolphins (Cunha et al., 2018) and in the present

study, two musks, galaxolide and galaxolidone, and two UV-filters, homosalate and octocrylene, were detected in oysters' tissues. The levels found were in general low. For instance, octocrylene levels (a compound highly lipophilic, therefore with high tendency to bioaccumulate) were in the same order of magnitude (low ng/g) of that reported for other seafood products (Cunha et al., 2018), resulting in a low potential health risk (Cunha et al., 2018). Galaxolide levels were also similar to those reported in mussels or fish fillets (Mottaleb et al., 2009; Picot Groz et al., 2014). To our knowledge, no studies on PCPs accumulation were carried out in oysters, highlighting the importance of the current study. Although higher concentrations of UV-filters were observed in summer in water and/or sediments, for musks, higher levels were detected in autumn, not showing a direct relationship with environmental levels.

Another group of pollutants that can also be related with wastewater effluent discharges is the group of FRs, as they have limited removal ratios in WWTPs (Aznar-Alemany et al., 2018). FRs are included in numerous materials, including household products, to reduce flammability. These substances were made to pursuit low biodegradability and durability, therefore, they are not easy to remove from wastewaters. Moreover, they have a high capacity to adsorb to sewage sludge (Covaci et al., 2011). In the present study, two classes of FRs were analysed, OPFRs and HFRs.

Regarding OPFRs, they were detected in all water samples, reaching higher levels in autumn. This peak could be attributed to the higher precipitation recorded in November 2016, causing untreated wastewater discharges through combined sewer overflows (CSO) inside the lagoon, instead of offshore through the treated sewage collector. This is supported by the higher nutrients (ammonium) levels in water observed in this season. Higher water movement could also have led to stronger sediment resuspension, potentially promoting the transfer of contaminants from the sediment exchangeable fraction to the water column. Higher concentrations of OPFRs in sediment samples in summer and autumn might be due to higher inputs from WWTPs. This was probably related to the higher number of tourists in the region during summer and also to the higher precipitation registered in autumn, leading to the discharge of a higher volume of untreated effluents. These values in sediments and water were lower or similar to those reported in sediments located near urban areas (Aznar-Alemany et al., 2018). Few OPFRs were also measured at low levels in oysters' tissues (TPPO, TDCPP and DCP) probably due to their presence in the surrounding environment, but with no clear seasonal trend. OPFRs are used as plasticisers, being widespread in the environment and they have been found in fish and mussels (e.g., Gao et al., 2014; Aznar-Alemany et al., 2018), but at much higher levels than those found in oysters in the present study.

Regarding HFRs, few compounds were detected, either in the sediment or in oyster tissues, showing a different behaviour from that observed for OPFRs. In general, higher values were registered in winter (oyster) and spring (sediments). In winter, bigger oysters with a slower growth rate could have led to a higher pollutant accumulation as discussed for PAHs, without a direct relationship with HFRs environmental levels. As HFRs have higher octanol-water partition coefficient and low water solubility, it is highly unlikely to detect halogenated compounds in water (Aznar-Alemany et al., 2018) so only OPFRs were analysed in water samples. Contrary to OPFRs, HFRs showed higher values in oyster tissues than in sediments, indicating that these compounds seem to have higher affinity for lipids with a tendency to accumulate in tissues. Since 2010 all PBDEs from tetra- to hepta-BDEs are regulated by the Stockholm Convention and are planned to be eliminated in the future. Moreover, since 2008, Dec-BDE no longer can be used in electronics and electrical applications in Europe (Vandermeersch et al., 2015). Yet, these compounds are still found in the environment and recent studies showed seafood contamination with PBDEs (Cruz et al., 2015; Aznar-Alemany et al., 2017). PBDEs are not bonded into plastics, but are simply blended within the polymers, enabling leaching out of materials (Aznar-Alemany et al., 2017). The most frequently found PBDE congeners are 47, 99, 100, 153, 154, 183 and 209, with BDE-47 the most concentrated in seafood (Vandermeersch et al., 2015). In the current oysters' tissues, although only two PBDEs congeners were detected, 47 and 209, the later was in a much higher concentration (up to 0.3 ng/g dw for the first and up to 160 ng/g dw for the second). The differences in PBDE profiles among species can be related with metabolic processes, age or lipid content (Vandermeersch et al., 2015). Levels found in oysters were in the same range of values reported for other seafood items available in European markets, and showed no health risk via seafood consumption (Aznar-Alemany et al., 2017).

Agriculture is another anthropogenic activity that occurs around the estuarine area of Canal de Mira, leading to the presence of pesticides in the environment and ultimately in seafood, such as oysters produced in this area. In general, pesticides, including pyrethroids insecticides, were not detected, neither in environmental samples (water and sediment) nor in oysters' tissues. However, a pyrethroid insecticide, cypermethrin, was detected in water in winter (S3), being unexpected as this compound presents low mobility in soils, low solubility in water and strong hydrophobicity (Palmquist et al., 2012). Another pyrethroid insecticide, permethrin, was also detected in oyster tissues, but only in summer and at low levels. This insecticide is commonly used as insect repellent, and the utilization in summer is likely explained by the increase in mosquitos' frequency during this season. Considering the low levels found, no major risk from seafood consumption is expected, although more research is certainly needed on this subject.

As previously mentioned, pollutants accumulation in seafood can pose a risk to human upon their consumption. In fact, European legislation has set several limits for the presence of pollutants in seafood. But, nowadays, much more pollutants can be present in the environment due to new human activities, posing potential new risk for which information is still not sufficient, namely for the so called pollutants of emerging concern. So, the assessment of the concentration of diverse families of pollutants, including both regulated and non-legislated pollutants, is needed to fully evaluate risks from seafood consumption. In the present work, the concentrations of a very high number of pollutants in oysters, whenever detected, were generally low. Considering such low levels, no major risk associated to the consumption of oysters produced in the selected aquaculture farm is, therefore, expected.

5. Conclusions

The present work assessed a wide range of emerging and persistent contaminants in oyster tissues produced in an aquaculture facility located in a relevant aquaculture area and in environmental samples (sediment and water).

The determination of a high number of pollutants belonging to different families, including those non- legislated for seafood, in oysters produced in a relevant area for aquaculture, evidenced the safety of such premium quality oysters for human consumption. The present study highlights that despite the detection of some contaminants in oysters, such as some PAHs, a few personal care product and a few flame retardants, in general, the concentrations were low, and do not represent a significant health risk due to seafood consumption. However, some seasonal variations were observed and, in some, a direct link with pollutant environmental levels could be established.

The management of human activities that have an impact on the marine environment, by integrating the concepts of environmental protection and sustainable use of resources is necessary to prevent any risk for human health. More studies like this one are highly relevant for the future management of resources contributing to a safe and healthy environment.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.02.280.

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