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Persistent organic pollutants in a marine bivalve on the Marennes–Oléron Bay and the Gironde Estuary (French Atlantic Coast)—Part 2: Potential biological effects

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HIGHLIGHTS

- Potentially contaminated sites were differentiated by a selection of key selected biomarkers.
- Active biomonitoring with juvenile oysters showed to be useful for this type of analysis.
- The presence of organic chemical contaminants may influence the defence responses in *C. gigas*.

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ABSTRACT

Contaminant effects on defence responses of ecologically and economically important organisms, such as the Pacific oyster *Crassostrea gigas*, are likely to influence their ability to resist infectious diseases, particularly at the young stages. The aim of this study was to explore the potential relationships between organic contaminants accumulated in the soft tissues of juvenile oysters, defence responses and physiological condition. Oysters were transplanted during summer and winter periods in different sites in the Marennes–Oléron Bay, the first area of oyster production in France, and in the Gironde Estuary, the biggest estuary in Occidental Europe. Amongst the battery of biochemical and physiological biomarkers applied in the present work [superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), malondyaldehyde (MDA), catecholase, laccase and lysozyme in gills, digestive glands, mantle and haemolymph, glycogen, proteins and lipids in the digestive gland and the condition index at the whole-organism level], MDA and lysozyme in the digestive gland and SOD, GPx and laccase in plasma contributed in order to significantly discriminate the sites in which oysters bioaccumulated different levels of heavy polycyclic aromatic hydrocarbons (HPAHs), polychlorobiphenyls (PCBs), polybromodiphenylethers (PBDEs), dichlorodiphenyltrichloroethanes (DDTs) and lindane. These results strengthen the hypothesis that it is possible to differentiate sites depending on their contamination levels and biological effects by carrying out studies with transplanted juvenile oysters. In addition, correlations were found between antioxidant and immune-defence responses, and PAH and DDT body burdens in the first area of oyster production in France (the Marennes–Oléron Bay) and where considerable oyster mortalities have been reported. This result suggests that the presence of organic chemical contaminants in the Marennes–Oléron Bay may influence defence responses in juveniles of *C. gigas*, and, therefore, could influence their ability to resist infectious diseases.

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1. Introduction

The proper assessment of chemical contamination in estuaries is crucial since these areas provide habitats for various organisms, support very high productivity and constitute nursery grounds for juveniles of several species (Johnston, 1981). In these areas, different countries have implemented monitoring programmes known as Mussel Watch Programmes (MWPs), in which chemical body burdens are analysed in naturally settled populations of marine bivalves as pollutant concentrations in their tissues faithfully reflect the environmental concentrations (Auffret et al., 2004; Livingstone, 1993).

Since chemical analyses cannot offer any type of indication of possible deleterious effects of contaminants on marine organisms, biomarker responses are also analysed in various monitoring programmes to

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evaluate the exposure to and toxic effects of contaminants (Allan et al., 2006; Cajaraville et al., 2000). Biomarker responses are biochemical, cellular, physiological, behavioural or energetic responses of the organisms measured in body fluids, cells, tissues or on whole animals (Bodin et al., 2004; Livingstone, 1993; Magni et al., 2006). They are considered as sensitive early-warning signals of deleterious effects of contaminants especially if they are analysed in an integrative approach, by measuring several biomarkers at the same time in the same animal (Lagadic et al., 1994).

Amongst the biomarker responses, defence mechanisms may be affected by the presence of contaminants in the environment (Lacoste et al., 2001; Perdue et al., 1981). A depletion of antioxidant enzymes such as superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6) and glutathione peroxidase (GPx; EC 1.11.1.9), may lead to the formation of reactive electrophile species such as malondialdehyde (MDA), which is considered as a marker for oxidative stress (Gerard-Monnier et al., 1998). A depletion of key enzymes involved in the humoral immune defence system in bivalves such as phenoloxidase (PO, EC 1.14.18.1; Söderhäll and Cerenius, 1998) and lysozyme (EC 3.2.1.17; Hagger et al., 2005), could influence the ability of the organism to resist infectious diseases. Glycogen, protein and lipid reserves can also be depleted in the presence of contaminants (Pellerin-Massicotte et al., 1994), and therefore are also considered as biomarkers (Lagadic et al., 1994). The condition index, defined as the ratio of flesh to shell dry weight (Lobel and Wright, 1982), is often used as the index endpoint to evaluate bivalve condition, and is considered as one of the best indicators of gross body state for environmental studies; thus, it can also reflect sub-lethal changes from a stressful xenobiotic exposure (Hyötylänen et al., 2002).

Previous studies in bivalve juveniles have shown that the responses of biomarkers such as SOD, CAT, GPx, MDA, catecholase, laccase and lysozyme can be modulated in experiments that are carried out in laboratory-controlled conditions with organic contaminants, and that these responses are tissue dependent (Luna-Acosta et al., 2011a, 2012). However, although laboratory studies may help to identify biomarkers of interest, a lack of validation may occur when using these biomarkers in the field with organisms from contaminated and reference sites. For example, external environmental factors, such as seawater temperature, may influence biomarker responses (Oliver and Fisher, 1995). In addition, internal factors such as body size may also influence biomarker responses (Packard and Boardman, 1999).

Therefore, laboratory results must be validated with in situ studies, especially through active biomonitoring. Active biomonitoring consists of using caged bivalves transplanted into sites with different levels of contamination. The main advantages of active biomonitoring over traditional MWPs are 1) the experimental control, which is achieved using bivalves of similar genetic and environmental stocks at all test sites, pre-selecting test animal size or age group and monitoring animals during the test; and 2) the possibility to examine both short- and long-term trends in contaminant distribution and their related effects (Besse et al., 2012; Galgani et al., 2011; Gunther et al., 1999).

The Marennes–Oléron Bay accounts for high levels of a wide range of anthropogenic contaminants such as heavy metals, polycyclic aromatic hydrocarbons (PAHs) and persistent organic pollutants (POPs) (Miramand et al., 2003; Munaron et al., 2006). In the Marennes–Oléron Bay, contaminants are mainly transported by the Charente River and, to a lesser extent, by the Seudre River (Miramand et al., 2003; Munaron et al., 2006; Luna-Acosta et al., 2010a). The bay is also influenced by inputs from the biggest estuary in occidental Europe, the Gironde Estuary (Miramand et al., 2003; Munaron et al., 2006). This bay is the first production site in France (Soletchnik et al., 1999) and one of the most important production sites worldwide (FAO, 2010) of the Pacific oyster *Crassostrea gigas* (Thunberg, 1753), the leading aquaculture product at the worldwide level (FAO, 2010). This marine bivalve is also an important species for ecosystem functioning (Gutierrez et al., 2003), and therefore, the maintenance of healthy populations of this economically and ecologically important organism is of considerable relevance. Like other bivalves, *C. gigas* has been extensively used in environmental risk assessment programmes, such as the French monitoring programme ROCCH (Réseau d'Observation de Contamination Chimique du Littoral), as pollutant concentrations in its tissues faithfully reflect the environmental concentrations.

To date, no transplantation studies with juvenile Pacific oysters analysing biomarker responses have been carried out, to the best of our knowledge, despite the fact that a very large number of summer mortalities, particularly of *C. gigas* juveniles, have become a widespread concern in the world in recent decades (Cheney et al., 2000; Garnier et al., 2007; Perdue et al., 1981). There is also growing evidence that contaminants may partly be responsible for the observed increase in disease and mortality in *C gigas*, by adversely affecting their defence mechanisms (Lacoste et al., 2001; Perdue et al., 1981).

Therefore, the main goals of this transplantation study were to evaluate 1) PAH and POP body burdens in transplanted juvenile oysters after a three-month transplantation period, in summer and winter, in different sites of the Marennes–Oléron Bay and the Gironde Estuary; 2) the potential effects of exposure to organic contaminants on antioxidant defence responses, immune defence responses, energy reserves and condition index in *C. gigas* juveniles; 3) the potential effects of external (e.g. temperature) and/or internal (e.g. body size) factors on juvenile oyster biomarker responses; and 4) the potential use of transplanted juvenile oysters to differentiate sites depending on their contamination levels and biological effects.

To achieve these goals, juvenile oysters bought in a hatchery, were transferred in summer and winter to Bouin (B), considered in previous studies as a reference site for chemical contamination (Geffard et al., 2002). Oysters were also transferred to different transplantation sites in the Marennes–Oléron Bay and the Gironde Estuary. After a three-month transplantation period, for each site and season, the body burdens of PAHs and POPs [polychlorobiphenyls (PCBs), polybromodimethylethers (PBDEs) and organochlorine pesticides (OCPs)], were analysed and the biochemical biomarker responses (SOD, CAT GPx, MDA, catecholase, laccase and lysozyme) were measured in different tissues (gills, digestive gland, mantle and haemolymph). Additionally, energy reserves (glycogen, proteins and lipids) in the digestive gland and the condition index were analysed and environmental data from French monitoring networks were collected.

2. Materials and methods

2.1. Study area and transplant design

One year-old juvenile Pacific oysters, *C. gigas*, of the same size (3–4 cm in height), were purchased from the oyster hatchery France Naissain, which is located in Vendée (France), at the beginning of each transplantation period, both in summer and winter. All animals were issued from the same breeding population in order to reduce genotype variability. In April 2008 and in October 2008, oysters were transplanted to Bouin (B; 46° 58′ 28″ N, 2° 00′ 02″ W), which is considered as a reference site (Fig. 1a) as it is located in a zone near the oyster hatchery, where the oysters were purchased, and because it possesses different seawater physicochemical characteristics in comparison to the transplantation zone (Geffard et al., 2002). In April 2008 and in October 2008, oysters were also transplanted into four other sites, considered as the transplantation sites: Boyard (BOY; 45° 58′ 0″ N, 1° 15′ 0″ W), under the double influence of inputs from the Gironde and the Charente estuaries; Les Palles (LP; 45° 58′ 0″ N, 1° 08′ 0″ W), highly influenced by inputs from the Charente River; Mus du Loup (ML; 45° 46′ 10″ N, 1° 08′ 30″ W), mildly influenced by inputs from the Seudre River; and Cordouan (C; 45° 35′ 11″ N, 1° 10′ 24″ W), highly influenced by inputs from the Gironde Estuary (Fig. 1b). Transplantation was carried out for a period of three months (90 days), from April to June 2008 (summer season) and

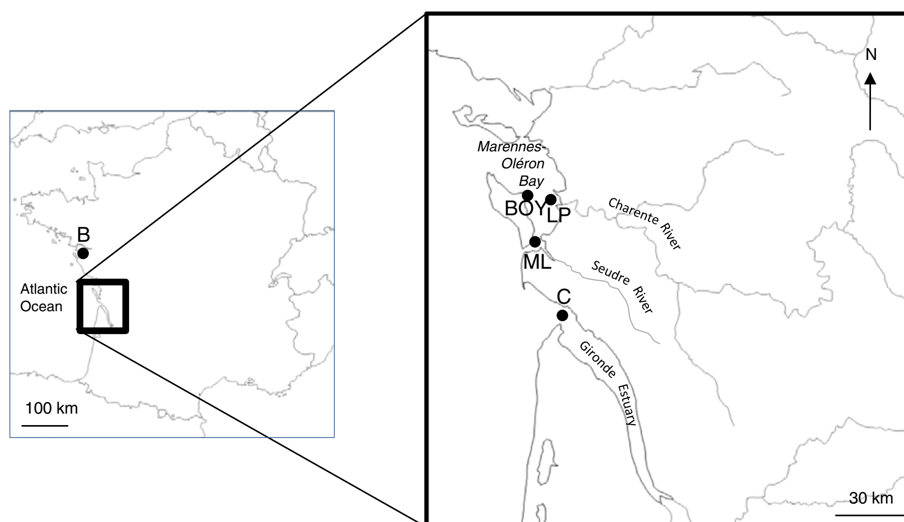

Fig. 1. Estuarine sampling areas in French Atlantic coast. Location of reference (B, Bouin) and transplantation sites: Boyard (BOY), Les Palles (LP), Mus du Loup (ML) in the Marennes–Oléron Bay and Cordouan (C) in the Gironde Estuary.

from October to December 2008 (winter season). For each site and season, a total of 100 individuals were placed in HDPE (high-density polyethylene) oyster bags, each measuring 1 m × 0.5 m with the same mesh size (2 cm). For each site, all individuals were transplanted in the same oyster bag and at the same location, and the oyster bags were placed on oyster aquaculture tables. These tables were placed at a height of 40 cm from the substrate and each one was located on the same hypsometric level (iso-altitude) of about 1 m, corresponding to low-tide water level (coefficient 90) in order to have an equivalent immersion time (<15% of the time over a tidal cycle), regardless of the geographic location. Three months after each transplantation period (in June 2008 and in December 2008) and for each site (B, BOY, LP, ML and C), five oysters were randomly collected for condition index and chemical analyses, and nine oysters were collected for biochemical biomarker and energetic reserve analyses. The oysters were transported in ice bags and processed immediately after their arrival in the laboratory.

2.2. Sample procedure

In the laboratory, the five oysters collected for condition index and chemical analyses were processed as follows. Soft tissues were removed from the shells, homogenised using an Ultra Turrax (T25 basic, IKA-WERKE) and a Thomas-Potter homogeniser (IKA-Labortechnik RW 20.n, size 0.13–0.18 mm), freeze-dried, weighed (dry weight; dw) for flesh dw, shell dw and condition index analyses ($n = 5$ per site and per season), and then pooled and frozen (−80 °C) for chemical analyses. For chemical analyses, three replicate tests were carried out per site and per season ($n = 3$, one sample replicate from five oysters analysed in triplicate, per site and per season).

For other biological analyses, the nine oysters collected for biochemical biomarker and energetic reserve analyses were processed as follows. The pooled gills, digestive glands, mantles and plasma of three oysters were used for each replicate sample, and three replicates were prepared per site and per season ($n = 9$, three sample replicates from three oysters each, per site and per season). To collect these samples, after opening the oyster shells by cutting off the adductor muscle, a quantity of haemolymph (ca. 0.3–0.5 mL for the three oysters) was withdrawn and centrifuged (260 ×g, 10 min, 4 °C) to separate the cellular fraction from the plasma. The gills, digestive gland and mantle were removed from the soft tissues and homogenised at 4 °C in 0.1 M Tris HCl buffer pH 7.0 (0.45 M NaCl, 26 mM $MgCl_2$, 10 mM $CaCl_2$; 0.5 mL of buffer per gram of fresh weight for the gills and the mantle, and 1 mL · g^{-1} of fresh weight for the digestive gland), using an Ultra Turrax (T25 basic, IKA-WERKE) and Thomas-Potter homogeniser (IKA-Labortechnik RW 20.n, size 0.13–0.18 mm). The homogenate was centrifuged at 10,000 ×g for 10 min at 4 °C. The resulting supernatant was collected for enzymatic analyses.

2.3. Chemical analysis

A total of 31 polyaromatic hydrocarbon (PAH), 10 polychlorobiphenyl (PCB), 4 polybromodimethylether (PBDE) and 13 organochlorine pesticide (OCP) compounds were quantified (concentrations of organic contaminants were expressed in μg kg^{-1} dw). Briefly, PAHs were measured using a gas chromatograph (HP 6890. Agilent technologies, Palo Alto, CA, USA) equipped with a splitless injector and coupled to a MSD 5975 mass spectrometer (Baumard et al., 1999). PCBs, PBDEs and OCPs were quantified using a gas chromatograph (HP 5890 Hewlett Packard, Palo Alto, Ca, USA) equipped with a splitless injector and coupled to an electron-capture detector (Thompson and Budzinski, 2000; Tapie et al., 2008) and confirmed using a gas chromatograph (Agilent Technologies 7890A) coupled to MS/MS (Agilent technologies 7000). Amongst the pesticides, lindane, 2,4′- and 4,4′-dichlorodiphenyltrichloroethanes (DDT), 2,4′- and 4,4′-dichlorodiphenyldichloroethanes (DDE), 2,4′- and 4,4′-dichlorodiphenyldichloroethylenes (DDD), hexachlorobenzene (HCB), heptachlor (HC) and trans-nonachlor (TNC) were quantified. In the text, we will refer to the sum of all DDTs and their metabolites (DDEs and DDDs) as 'DDTs' and to the sum of HCB, HC and TNC as 'HCB + HC + TNC'. More details on the methodology and quality control are described by Luna-Acosta et al. (2015–in this volume).

2.4. Biological analysis

2.4.1. Superoxide dismutase (SOD) assay

SOD was determined by an indirect method (Therond et al., 1996), based on the competition of SOD with iodonitrotetrazolium (INT) for the dismutation of the superoxide anion (O_2^-). In the presence of O_2^-, INT is reduced into a red formazan dye that can be measured at 505 nm at 25 °C (Ransod SD 125 Kit, Randox, France). One unit of SOD is defined as the amount of enzyme that promotes a 50% decrease in the rate of INT reduction.

2.4.2. Catalase (CAT) assay

Catalase activity was determined according to the method of Fossati et al. (1980). This assay method is based on the measurement of the amount of hydrogen peroxide substrate remaining after the action of

catalase. First, catalase converts hydrogen peroxide to water and oxygen, and then this enzymatic reaction is stopped with sodium azide. An aliquot of the reaction mix is then assayed for the amount of hydrogen peroxide remaining, using a colorimetric method (Catalase Kit CAT-100, Sigma).

2.4.3. Glutathione peroxidase (GPx) assay

GPx activity was determined according to the method of Paglia and Valentine (1967). In the presence of glutathione reductase and substrates (i.e. reduced glutathione and cumene hydroperoxide), the decrease in absorbance at 340 nm is proportional to the reduction in the amount of glutathione oxidised by NADPH, H+ (Ransel RS 504 Kit, Randox, France). One unit of GPx oxidises 1 nmol of NADPH ($\varepsilon = 6.22$ mM^{-1} cm^{-1}) per minute.

2.4.4. Phenoloxidase (PO, catecholase- and laccase-type) assay

Catecholase- and laccase-type PO activities have been detected in *C. gigas* (Luna-Acosta et al., 2010b). Spectrophotometric measurements of these PO activities were made as described by Luna-Acosta et al. (2010b). Assays were carried out in triplicate for each sample. Briefly, the catecholase-type PO assay was carried out in the presence of 100 mM dopamine and 0.1 M Tris HCl buffer pH 7.0 (0.45 M NaCl, 26 mM $MgCl_2$, 10 mM $CaCl_2$; Sigma–Aldrich, France). Catecholase activity was followed by monitoring the increase of absorbance at 490 nm for 4 h. The laccase assay was carried out in the presence of 50 mM of p-phenylenediamine (PPD), diluted in methanol, and 0.1 M Tris HCl buffer pH 7.0. Laccase activity was followed by monitoring the increase in absorbance at 420 nm for 2 h. Non-enzymatic oxidation of the substrate was followed in wells without the oyster sample and subtracted from the oxidation of the substrate with an oyster sample. One unit of catecholase specific activity and one unit of laccase specific activity correspond to the amount of enzyme that catalyses the production of 1 μmol of product ($\varepsilon = 3{,}300$ and $43{,}160$ $M^{-1} \cdot cm^{-1}$, respectively), per minute and per milligram of protein (Eggert et al., 1996; Espin et al., 1995).

2.4.5. Lysozyme assay

The lysozyme assay was performed in triplicate for each sample and compared against hen egg-white lysozyme standards (0.4–40 $\mu g \cdot mL^{-1}$) in the presence of *Micrococcus lysodeikticus* (Sigma–Aldrich, France). One unit of lysozyme corresponds to the amount of enzyme that diminishes the absorbance at 450 nm of 0.001 per min, at pH 7.0, at 25 °C (Soudant et al., 2004).

2.4.6. Malondialdehyde (MDA) assay

Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds, of which the most abundant is MDA (Gerard-Monnier et al., 1998). The method used to determine MDA levels in the gills and the digestive gland (MDA-586, Oxis Research, Portland, OR, USA) was based on the reaction of a chromogenic reagent, N-methyl-2-phenylindole (NMPI) with MDA at 45 °C. One molecule of MDA reacts with two molecules of NMPI to yield a stable carbocyanine dye, which has a maximum absorption at 586 nm. The MDA in the sample was determined from the absorbance of the sample at 586 nm, and the standard curve was prepared using the MDA standard provided in the kit.

2.4.7. Glycogen assay

Digestive gland samples were freeze-dried and weighed before determining the glycogen, lipid and protein contents. The glycogen content (precipitated with absolute ethanol) was quantified using the phenol–sulphuric method, as described by Dubois et al. (1956). Known glucose standards were processed in the same way as the samples and used to construct a calibration curve.

2.4.8. Lipid assay

Lipids were extracted according to the procedure of Folch et al. (1956). A double static extraction (2×12 h) was carried out on the rehydrated samples with a chloroform/methanol mix (1:2 then 2:1, v/v) in polytetrafluoroethylene (PTFE)-capped tubes held at 4 °C under a nitrogen atmosphere. Following the addition of a salt solution (NaCl 1%), the crude extract was separated into two phases. The lower chloroform phase containing lipids was washed gently with a new volume of the upper phase. After centrifugation for 10 min at 3000 ×g at 10 °C and subsequent decantation, it was recovered and stored at −20 °C until analysis. The total lipids were measured using a Iatroscan–Chromarod system [thin-layer chromatography–flame ionisation detection (TLC–FID)] connected to a Shimadzu CR3A integrator. Five successive volumes (1 μL) of a concentrated extract aliquot were spotted onto a Chromarod SIII silica rod with a 2 μL Hamilton microsyringe. After drying in a desiccator, the rod was read directly by the Iatroscan without any development. A calibration curve based on the total-lipid extract from cod-liver oil, with similar lipid characteristics to oyster tissues, was used to calculate the lipid concentration of the sample extracts.

2.4.9. Protein assay

The protein concentration was measured according to the Lowry method with slight modifications, using bicinchoninic acid and copper sulphate 4% (Smith et al., 1985). Serum albumin was used as the standard (Sigma–Aldrich, France). All enzyme activities were expressed in relation to protein concentration.

2.4.10. Condition index

The condition index in juvenile oysters was calculated using the method of Lobel and Wright (1982), and expressed in mg flesh dw g^{-1} shell dw.

2.5. Statistical analysis

All values are reported as the mean ± standard deviation (SD; $n = 5$ per site and season for flesh dw, shell dw and condition index analyses; $n = 3$ per site and per season for chemical analyses and $n = 9$ per site and season for biochemical biomarkers and energetic reserve analyses). Values were tested for normality (Shapiro test) and homogeneity of variances (Bartlett test). In some cases (HPAHs, PCBs, PBDEs, DDTs, lindane and HCB + HC + TNC body burdens, catecholase, laccase, lysozyme in gills, GPx, MDA, lysozyme, proteins and lipids in the digestive gland, SOD, GPx, catecholase, and lysozyme in mantle and SOD, CAT, laccase and lysozyme in plasma), logarithmic transformations (log10) were used to meet the underlying assumptions of normality and homogeneity of variances. As body size and/or gross body state may influence the biomarker responses (Packard and Boardman, 1999), two-way MANCOVA (covariate: flesh dw, shell dw or condition index) was carried out to evaluate these possible effects, with site and season as fixed factors (Zar, 1984). After verifying that the tested covariates had no significant effect on the biomarker responses ($p > 0.05$), a two-way MANOVA was used, followed by Tukey's HSD test, with site and season as fixed factors. In addition, a discriminant analysis (DA), using three different groups of sites depending on their degree of contamination in order to test which biomarker responses significantly contributed towards discriminating sites between these three groups, and the ordination technique of redundancy analysis (RDA), using forward selection of explanatory variables in order to test which contaminants had a significant effect on the observed biomarker responses were performed to evaluate the response of biomarkers to chemical analyses. Data were centred and standardised before analysis (Leps and Smilauer, 1999). The significance of the relationships between biomarker responses and contaminant body burdens were tested using a global Monte Carlo permutation test. Statistical analyses of data were performed using the software STATISTICA 7.0, with the exception

of DA and RDA, which were performed using the software XLSTAT 5.0. Significance was achieved at $p < 0.05$.

3. Results and discussion

3.1. PAH and POP body burdens in transplanted juvenile oysters

In the present study, over the different sampling stations (i.e. reference and transplantation sites) and seasons (i.e. summer and winter), body burdens were 27 ± 1–68 ± 19 μg kg^{-1} dw for LPAHs, 35 ± 7–159 ± 34 μg kg^{-1} dw for HPAHs, 15 ± 1–89 ± 39 μg kg^{-1} dw for PCBs, 0.4 ± 0.0–2.9 ± 0.7 μg kg^{-1} dw for PBDEs, 2 ± 0–15 ± 0 μg kg^{-1} dw for DDTs, 0.1 ± 0.0–2.2 ± 1.1 μg kg^{-1} dw for lindane, and 0.7 ± 0.0–3.5 ± 1.0 μg kg^{-1} dw HCB + HC + TNC (Table 1; Supplementary Tables 1, 2 and 3). Transplanted oysters with the highest HPAH and DDT body burdens were observed in LP in summer and in LP, BOY and ML in winter (Table 1). Surprisingly, transplanted oysters with the highest PCB, PBDE and lindane body burdens were observed in B, which has been considered as a reference site for chemical contamination in previous studies (e.g. Geffard et al., 2002; Table 1). Results of the present study reveal that B should no longer be considered as a reference site and show that special care must be taken in future studies. Previous authors have pointed out the difficulties encountered in establishing a reference site that is not polluted (Davies and Vethaak, 2012), and some authors have even argued that there is no such thing as a pristine environment (Clark, 1982). Thus, B remains an interesting site in the present study, as it is located in a zone near the oyster hatchery, where oyster were purchased, and because it possesses different seawater physicochemical characteristics in comparison to the transplantation zone (Geffard et al., 2002). Moreover, although it is difficult to determine the normal range of biomarker responses in the present study, the chemical compounds that were analysed in the present study are not naturally present in animals, so their presence unequivocally indicates the presence of environmental pollution and, therefore, the findings of the present study are not compromised, as relationships between biomarkers response and exposure can still be determined.

Another interesting result was that oysters from C, located in the Gironde Estuary, did not have the highest body burdens for the organic contaminants analysed in the present study (Table 1), suggesting that the Marennes–Oléron Bay is not subjected to chronic pollution by PAHs or POPs from the Gironde watershed. These results were not expected, as the Marennes–Oléron Bay is known to be influenced by historic polymetallic pollution of the Gironde Estuary, especially cadmium pollution (Pigeot et al., 2006; Strady et al., 2011), and therefore, the bay was expected to be influenced by other contaminants, such as PAHs and POPs, of the Gironde Estuary (Laroche et al., 2013). These results also suggest the presence of other contaminant sources in the Marennes–Oléron Bay, such as local inputs from agricultural or industrial activities, as more deeply discussed in Luna-Acosta et al. (2015–in this volume) and are in agreement with other studies (e.g. Greenfield et al., 2014; Hédouin et al., 2011; Orbea and Cajaraville, 2006) that show how transplant experiments with bivalves could be useful for assessing the origin of chemical pollution.

3.2. Potential effects of exposure to organic contaminants on antioxidant defence responses, immune defence responses, energy reserves and condition index in C. gigas juveniles

General trends in biomarker responses for oysters from transplantation sites in the Marennes–Oléron Bay and the Gironde Estuary are presented in this paragraph in comparison to biomarker responses in oysters from B (Table 2). The SOD levels were, generally, significantly higher, especially in LP and BOY, except when they were measured in the gills (Table 2). CAT levels were also significantly higher when measured in the digestive gland or in plasma (Table 2). GPx levels were significantly lower or higher, depending on the analysed tissue and season (Table 2). MDA levels were, generally, significantly higher in the gills and in the digestive gland, especially in winter (Table 2). Catecholase levels were higher when measured in the gills and the digestive gland, but lower when measured in plasma, especially in summer (Table 2). Laccase levels were lower, independently of the season (Table 2), and lysozyme levels were lower, except when measured in winter in the digestive gland (Table 2). Thus, as a general trend, higher levels of SOD, CAT, GPx, MDA, catecholase, laccase and lysozyme were observed in winter.

Humoral immune defence responses from the present study are in agreement with previous studies that have reported lower immune defences in summer (Samain et al., 2007). However, the latter has generally been attributed to the reproductive cycle of bivalves in summer (Samain et al., 2007), but in the present study, the oysters were juveniles. Results on MDA levels in the present study are in agreement with previous studies in bivalves, which have reported higher oxidative stress in winter, but results on SOD, CAT and GPx are in disagreement with previous studies in bivalves, having reported that, in general, antioxidant defences in bivalves are lower in winter (Manduzio et al., 2004). Thus, factors other than the seasonality are likely to affect biomarker responses in the present study.

Concerning other biomarker responses analysed in the present study, glycogen, lipids and proteins in the digestive gland are generally accounted for as 5–15%, 60–70% and 10–20% of the total energy reserves, respectively (Fig. 2b, c), which also varied depending on the season and site (Fig. 2a). A significant decrease in lipid content was observed between summer and winter in oysters from B (Fig. 2a). When analysing results from other sites, significant low levels in glycogen in oysters from BOY, LP and C were concomitant with significant high levels in lipids and proteins. Low glycogen levels could be

Table 1
Contaminant contents in oyster's flesh at two seasons after 3 months of transplantation. Mean ± SD (μg kg^{-1} dw), $n = 3$ (i.e. 3 replicates from 1 pool of 5 oysters). B: Bouin; BOY: Boyard; LP: Les Palles; ML: Mus du Loup; C: Cordouan; dw: dry weight; nd: not detected. PAHs: polycyclic aromatic hydrocarbons; LPAHs: low molecular weight PAHs; HPAHs: high molecular weight PAHs; PCBs: polychlorobiphenyls; PBDEs: polybromodimethylethers; DDTs (DDT and metabolites: dichlorodiphenyltrichloroethanes (2,4′-DDT and 4,4′-DDT) + dichlorodiphenyldichloroethylenes (2,4′-DDE and 4,4′-DDE) + dichlorodiphenyldichloroethanes (2,4′-DDD and 4,4′-DDD)); HCB: hexachlorobenzene; HC: heptachlor; TNC: trans-nonachlor.

Contaminants	Concentration (μg kg^{-1} dw)									
	Summer					Winter				
	B	BOY	LP	ML	C	B	BOY	LP	ML	C
LPAHs	68 ± 6	58 ± 9	47 ± 14	27 ± 1	45 ± 25	69 ± 16	68 ± 19	60 ± 12	41 ± 5	44 ± 5
HPAHs	60 ± 1	81 ± 10	141 ± 11	54 ± 10	35 ± 7	87 ± 10	167 ± 25	145 ± 3	159 ± 34	76 ± 12
PCBs	81 ± 9	30 ± 3	23 ± 4	15 ± 1	39 ± 12	89 ± 39	47 ± 3	30 ± 3	17 ± 6	44 ± 4
PBDEs	0.9 ± 0.0	0.4 ± 0.4	0.4 ± 0.3	0.4 ± 0.0	0.9 ± 0.2	2.9 ± 0.7	0.6 ± 0.3	1.0 ± 0.7	0.6 ± 0.1	0.5 ± 0.1
DDTs	6 ± 1	8 ± 1	6 ± 1	2 ± 0	3 ± 0	6 ± 3	15 ± 0	10 ± 1	4 ± 0	8 ± 2
Lindane	2.1 ± 0.4	0.6 ± 0.1	0.4 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	2.2 ± 1.1	0.5 ± 0.2	1.3 ± 0.3	0.6 ± 0.1	0.7 ± 0.4
HCB + HC + TNC	2.2 ± 0.4	2.4 ± 0.9	2.0 ± 0.3	0.8 ± 0.0	0.8 ± 0.0	1.8 ± 0.8	3.5 ± 1.0	4.1 ± 1.9	0.7 ± 0.0	1.9 ± 0.1

Table 2

Biomarker responses in the gills, digestive gland, mantle and plasma in *C. gigas* from reference, i.e. Bouin, and transplantation sites, i.e. Boyard, Les Palles, Mus du Loup and Cordouan, in summer and winter. Data are expressed as mean ± SD (n = 3 pools from 3 individuals). For clarity of results, only significant differences between reference and transplantation sites are shown.

Biomarkers		Tissue	Summer					Winter				
			Bouin	Boyard	Les Palles	Mus du Loup	Cordouan	Bouin	Boyard	Les Palles	Mus du Loup	Cordouan
Defence	Superoxide dismutase (U mg prot^{-1})	Gills	120 ± 11	82 ± 7***	95 ± 9**	94 ± 14**	72 ± 10***	106 ± 6	78 ± 5***	108 ± 11	111 ± 15	70 ± 5***
		Digestive gland	22 ± 6	25 ± 6	62 ± 12***	31 ± 8	44 ± 5**	25 ± 5	73 ± 9***	19 ± 2	36 ± 11	51 ± 3**
		Mantle	240 ± 8	205 ± 9***	312 ± 4***	309 ± 11***	54 ± 8***	238 ± 11	288 ± 4***	329 ± 13***	185 ± 3***	25 ± 7***
		Plasma	44 ± 10	168 ± 58***	113 ± 11*	63 ± 2	57 ± 13	26 ± 8	138 ± 47***	80 ± 12	115 ± 9**	31 ± 8
	Catalase (U mg prot^{-1})	Digestive gland	2.4 ± 0.2[a]	2.3 ± 0.2	4.5 ± 0.1***	2.4 ± 0.2	4.4 ± 0.7***	0.8 ± 0.1[b]	1.0 ± 0.4	1.1 ± 0.9	2.5 ± 0.3**	0.0 ± 0.5***
		Plasma	12.4 ± 0.5	10.0 ± 6.2	28.4 ± 6.2***	11.5 ± 3.7	12.0 ± 0.1	13.7 ± 4.7	45.8 ± 6.5***	20.4 ± 7.5	18.9 ± 11.0	1.9 ± 0.7***
	Glutathion peroxidase (nmol mg prot^{-1})	Gills	25 ± 7	12 ± 2*	33 ± 5	9 ± 4*	48 ± 5***	17 ± 10	28 ± 8	11 ± 2	39 ± 3**	59 ± 6***
		Digestive gland	40 ± 8[a]	42 ± 8	12 ± 7***	64 ± 15	32 ± 13	78 ± 3[b]	19 ± 7***	88 ± 33	0 ± 18***	149 ± 29**
		Plasma	56 ± 7	22 ± 5***	37 ± 11*	38 ± 6**	32 ± 4**	48 ± 9	19 ± 7***	34 ± 5**	41 ± 9	41 ± 5
Damage	Malondialdehyde (μmol mg prot^{-1})	Gills	6.6 ± 0.9[a]	9.5 ± 2.2	10.9 ± 1.5*	7.4 ± 1.0	2.0 ± 0.4***	3.4 ± 1.2[b]	9.8 ± 1.1***	38.2 ± 8.1***	23.1 ± 1.8***	10.6 ± 1.7***
		Digestive gland	48 ± 16	71 ± 3*	66 ± 10	72 ± 17	39 ± 18	34 ± 13	94 ± 6***	74 ± 11**	52 ± 1*	78 ± 7***
Immuno-modulation	Catecholase (μmol min^{-1} mg prot^{-1})	Gills	32 ± 4[a]	38 ± 1	32 ± 2	43 ± 3	34 ± 9	22 ± 1[b]	17 ± 2	21 ± 2	26 ± 4	38 ± 7**
		Digestive gland	33 ± 2	95 ± 7***	57 ± 6***	69 ± 3***	49 ± 10**	33 ± 3	50 ± 4***	83 ± 5***	92 ± 5***	70 ± 7***
		Mantle	32 ± 6	48 ± 15	69 ± 31	126 ± 32***	54 ± 18	71 ± 3	35 ± 1	38 ± 23	46 ± 7	0 ± 9***
		Plasma	52 ± 8	28 ± 6*	34 ± 7	26 ± 7**	31 ± 8*	53 ± 7	85 ± 10***	59 ± 4	88 ± 13***	30 ± 5*
	Laccase (μmol min^{-1} mg prot^{-1})	Gills	9.1 ± 0.9	7.0 ± 0.8	6.3 ± 0.9*	8.1 ± 0.4	8.2 ± 2.5	8.8 ± 0.8	5.4 ± 0.6**	8.0 ± 0.5	6.8 ± 0.3	13.9 ± 2.4**
		Digestive gland	4.9 ± 0.3[a]	0.7 ± 0.5***	2.0 ± 0.1***	4.0 ± 0.6	5.4 ± 1.6	7.5 ± 1.0[b]	3.2 ± 0.5***	5.5 ± 0.3*	4.9 ± 0.8**	3.7 ± 0.4***
		Mantle	10.8 ± 0.7[a]	2.4 ± 1.0***	4.7 ± 0.6***	4.0 ± 0.8***	10.1 ± 1.0	13.6 ± 1.7[b]	4.1 ± 0.5***	4.3 ± 0.3***	3.7 ± 0.6***	6.3 ± 1.1***
		Plasma	11.9 ± 0.5[a]	8.9 ± 0.6***	2.2 ± 0.2***	1.9 ± 0.8***	2.8 ± 1.5***	8.7 ± 0.9[b]	3.3 ± 1.4***	1.1 ± 0.2***	1.1 ± 0.2***	2.6 ± 0.4***
	Lysozyme (U mg prot^{-1})	Gills	22 ± 1[a]	17 ± 2	24 ± 2	20 ± 4	9 ± 3***	14 ± 2[b]	8 ± 2***	15 ± 2	8 ± 1***	12 ± 2
		Digestive gland	48 ± 6[a]	30 ± 5***	7 ± 1***	47 ± 2	8 ± 1***	25 ± 1[b]	33 ± 2**	34 ± 2**	33 ± 2*	14 ± 4***
		Mantle	91 ± 13	103 ± 7	71 ± 5	65 ± 1*	68 ± 11	76 ± 2	68 ± 15	68 ± 5	90 ± 17	72 ± 15
		Plasma	51 ± 3[a]	44 ± 1**	35 ± 1**	33 ± 1**	14 ± 1***	42 ± 3[b]	29 ± 1**	42 ± 4	40 ± 1	37 ± 1*

Significant differences with Bouin: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Superscript letters indicate significant ($p < 0.05$) differences within seasons in Bouin.

Table 3
Physico-chemical and biological parameters of the reference (Bouin, B) and the transplantion sites (Boyard, BOY; Les Palles, LP; Mus du Loup, ML; Cordouan: C) in summer (June) and winter (December), 2008.

	Summer					Winter				
	B	BOY	LP	ML	C	B	BOY	LP	ML	C
Physicochemical parameters										
Temperature (°C)[a]	18 ± 2	19 ± 2	20 ± 2	20 ± 2	21 ± 2	9 ± 2	9 ± 2	8 ± 2	10 ± 3	8 ± 3
Salinity[a]	33 ± 1	33 ± 1	33 ± 1	33 ± 1	33 ± 2	31 ± 2	32 ± 2	31 ± 3	30 ± 3	32 ± 3
Turbidity (NTU)[a]	4 ± 5	6 ± 4	4 ± 1	10 ± 8	4 ± 6	34 ± 27	22 ± 11	12 ± 8	14 ± 12	10 ± 11
Biological parameters										
Chlorophyll *a* (μg l^{-1})	1.6 ± 1.91 [a]	2.8[b]	5.4 ± 3.3 [a]	2.6[b]	3.1 [a]	1.1 ± 0.9 [a]	1.1 [b]	0.8 ± 0.4[a]	0.6[b]	1.2 [a]
Phytoplancton (cells l^{-1})[a]										
Cryptophyceae (*Cryptophyceae*)	10^{5-6}	–	–	–	–	–	–	–	–	–
Asterionellopsis glacialis (*Diatomophyceae*)	10^{5-6}	–	–	–	–	–	–	–	–	–
Chaetoceros sp. (*Diatomophyceae*)	10^{5-6}	10^{5-6}	10^{5-6}	10^{5-6}	10^{5-6}	–	10^{3-4}	10^{3-4}	10^{3-4}	–
Cylindrotecha closterium (*Diatomophyceae*)	–	–	–	–	–	10^{3-4}	10^{3-4}	10^{3-4}	10^{3-4}	–
Guinardia delicatula (*Diatomophyceae*)	–	–	–	–	10^{5-6}	–	–	–	–	–
Leptocylindrus sp. (*Diatomophyceae*)	10^{5-6}	10^{5-6}	10^{5-6}	10^{5-6}	10^{5-6}	–	–	–	–	–
Nitzchia longissima (*Diatomophyceae*)	10^{5-6}	10^{5-6}	10^{5-6}	10^{5-6}	10^{5-6}	–	–	–	–	10^{3-4}
Paralia sulcata (*Diatomophyceae*)	–	10^{5-6}	10^{5-6}	10^{5-6}	10^{3-4}	–	10^{3-4}	10^{3-4}	10^{3-4}	10^{5-6}
Skeletonema costatum (*Diatomophyceae*)	10^{5-6}	–	–	–	10^{5-6}	10^{3-4}	$<10^{3}$	$<10^{3}$	$<10^{3}$	–
Thalassiosira sp. (*Diatomophyceae*)	–	–	–	–	10^{5-6}	10^{3-4}	–	–	–	–
Gymonodiniaceae (*Dinophyceae*)	–	–	–	–	10^{5-6}	–	–	–	–	–
Phytoplancton producing phycotoxins (cells l^{-1})[a]					–					
Alexandrium sp. (*Diatomophyceae*)	$\leq 10^{2}$	$\leq 10^{1}$	$\leq 10^{1}$	$\leq 10^{1}$	10^{3-4}	$\leq 10^{1}$	$\leq 10^{1}$	$\leq 10^{1}$	$\leq 10^{1}$	$\leq 10^{2}$
Dinophysis sp. (*Dinophyceae*)	$\leq 10^{2}$	$\leq 10^{2}$	$\leq 10^{1}$	$\leq 10^{1}$	$\leq 10^{1}$	$\leq 10^{1}$	$\leq 10^{1}$	$\leq 10^{1}$	$\leq 10^{1}$	$\leq 10^{1}$
Pseudo–nitzchia sp. (*Diatomophyceae*)	$\leq 10^{4}$	$\leq 10^{3}$	$\leq 10^{2}$	$\leq 10^{2}$	$\leq 10^{4}$	$\leq 10^{1}$	$\leq 10^{2}$	$\leq 10^{1}$	$\leq 10^{1}$	$\leq 10^{1}$
Phycotoxins[a]										
Amnesic shellfish poisoning (ASP)	–	–	–	–	–	–	–	–	–	–
Diarrheic shellfish poisoning (DSP)	–	–	–	–	–	–	–	–	–	–
Paralytic shellfish poisoning (PSP)	–	–	–	–	–	–	–	–	–	–
Faecal contamination (*E. coli* 100^{-1} g^{-1} ILC)[a]	<230	<230	<230	<230 to 4 600	<230	<230	<230	<230	<230	<230

"–": not detected.
ILC: intervalvular liquid content.
[a] Mean values ± SD adapted from 2000 to 2006 data of the website "Site Ifremer consacré à l'environnement littoral": http://www.ifremer.fr/envlit/.
[b] Mean values of 2008, collected by the French network Razlec (Ifremer, http://www.ifremer.fr/lerpc/reswaterx/hydro/resul_hydro.htm). Values correspond to the mean of June and December of 2008 of two measurements carried out per month at the bottom and at the top of the water surface from ML and sites near BOY and LP.

attributed to an exposure of the organism to high levels of contaminants (Pellerin-Massicotte et al., 1994) or the use of glycogen reserves in oysters for the synthesis of lipids and/or proteins for gametogenesis (De La Parra et al., 2005; Gabbott, 1975). High levels of glycogen in ML in summer, concomitant with low levels of lipids and proteins, could suggest a delay in gametogenesis in this site (Gauthier-Clerc et al., 2002), or that organisms in this site were not exposed to high levels of contaminants. When comparing these results to the total flesh dw, the digestive gland weight in oysters from BOY, LP, ML and C was lower in summer than in winter (Fig. 2b), and this could be associated to gametogenesis (De La Parra et al., 2005). However, further studies should be carried out to verify this hypothesis.

In the present study, higher condition index values were observed in summer in comparison to winter (Fig. 3c). These results are in agreement with previous studies in mussels and oysters (Bodin et al., 2004; Soletchnik et al., 2006). Interestingly, significant differences between the reference and transplantation sites were observed in summer, but not in winter. In summer, a significant increase in the condition index was observed in a North–South gradient, with higher values observed in C. In comparison to the condition index of oysters from B, the condition index was ~1.3, ~1.3, ~1.7 and ~2 times significantly higher in BOY, LP, ML and C, respectively. This increase in the condition index was not due to an important increase in shell dw (Fig. 3b), but to an important increase in flesh dw (Fig. 3a). However, no clear differences in contaminant body burdens between sites were observed in a North–South gradient.

To further evaluate potential relationships between biomarker responses and contaminant body burdens, a DA analysis was carried out, dividing sites into three groups based on contaminant body burdens (Table 1). Group 1 corresponded to B in summer and winter (Bs and Bw, respectively), which turned out to be the most contaminated site concerning PCB, PBDE and lindane contamination, group 2 corresponded to LP in summer and winter (LPs and LPw, respectively), BOY in winter (BOYw) and ML in winter (MLw), which was the most contaminated sites concerning HPAH and DDT contamination (Table 1), and group 3 corresponded to the remaining sites [BOY and ML in summer (BOYs and MLs, respectively), and C in summer and winter (Cs and Cw, respectively)]. Biomarker responses that significantly ($p < 0.001$) contributed towards discriminating the sites into these three groups were MDA in gills, SOD, GPx, MDA, lysozyme and proteins in the digestive gland, SOD in the mantle, SOD, GPx and laccase in plasma and the condition index. RDA analysis was carried out with these biomarkers.

The results of the RDA analysis are presented in the tri-plot ordination diagram in Fig. 4. Amongst the contaminants that were analysed, HPAHs, PCBs, PBDEs, DDTs and lindane had a significant effect on the observed biomarker responses. The first two axes of the RDA analysis accounted for 77% of the overall variability in the data. Therefore, the other axes were neglected because they did not provide significant additional information. The first RDA axis (horizontal) accounted for 42% of the total, and HPAHs and DDTs strongly correlated with the first axis. In addition, MDA in the gills and the digestive gland, SOD in mantle, and SOD and GPx in plasma correlated positively with the first axis, and the condition index correlated negatively with the first axis (Fig. 4a).

The second RDA axis (vertical) accounted for 35% of the total variability of the data, and the levels of PCBs, PBDEs and lindane strongly correlated with the second axis. In addition, GPx in the digestive gland and laccase in the plasma and mantle correlated positively with the second axis, and SOD in the digestive gland correlated negatively with the second axis (Fig. 4a).

Thus, the results of the RDA analysis suggest that HPAH and/or DDT contamination increase SOD activity in the mantle, whereas SOD and GPx activities in plasma and PCB, PBDE and/or lindane contamination increase GPx activities in the digestive gland (Fig. 4a). The observed

Fig. 2. Energy reserves (glycogen, lipids, proteins) in the digestive gland of C. gigas. a. Energy reserves from reference site (Bouin, white bars), and transplantation sites (Boyard — light grey bars, Les Palles — medium grey bars, Mus du Loup — dark grey bars and Cordouan — black bars), in summer (S) and in winter (W). Data are expressed as mean ± SD. For clarity of results, only significant differences between reference and transplantation sites are shown; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Superscript letters indicate significant ($p < 0.05$) differences between B samples from summer and winter. b. Levels of energy reserves (glycogen in white, lipids in black, proteins in grey) are expressed in percentage (%) relative to the total flesh dry weight. c. Levels of energy reserves (glycogen in white, lipids in black, proteins in grey) are presented in percentage (%) relative to the total fresh dry weight, with the total fresh dry weight standardized for all sites and seasons to 100%. B: Bouin, BOY: Boyard, LP: Les Palles, ML: Mus du Loup, C: Cordouan, S: Summer, W: Winter.

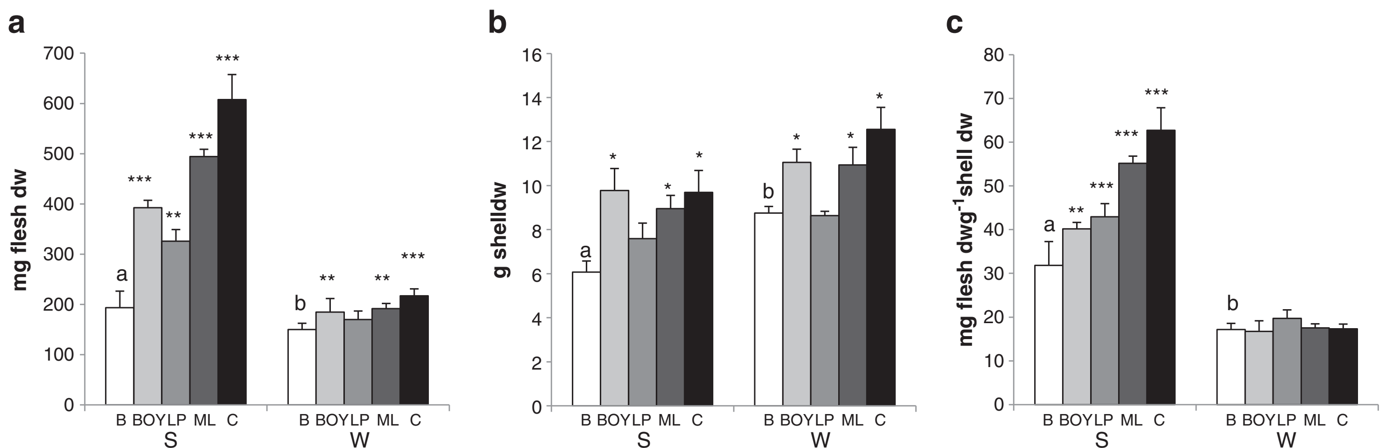

Fig. 3. Fig. 3 Flesh dry weight (a), shell dry weight (b) and condition index (c) in C. gigas from reference site (Bouin, white bars), and transplantation sites (Boyard — light grey bars, Les Palles — medium grey bars, Mus du Loup — dark grey bars and Cordouan — black bars), in summer (S) and in winter (W). Data are expressed as mean ± SD. For clarity of results, only significant differences between reference and transplantation sites are shown; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Superscript letters indicate significant ($p < 0.05$) differences between B samples from summer and winter. B: Bouin, BOY: Boyard, LP: Les Palles, ML: Mus du Loup, C: Cordouan, S: Summer, W: Winter.

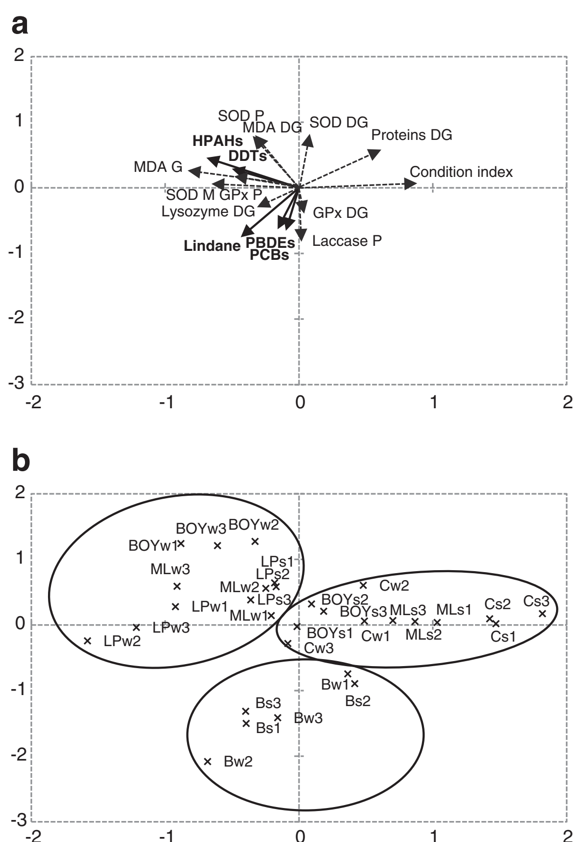

Fig. 4. Redundancy analysis (RDA) ordination diagram with (a) contaminant contents (straight arrows), and biochemical parameters (dotted arrows) and (b) sampling sites. First axis is horizontal, second axis is vertical. The first axis displays 42% of the remaining variation in contaminants in oysters, the second axis another 35%. Only biomarkers that significantly ($p < 0.05$) contributed to discriminate sites depending on their degree of contamination were used to carry out the RDA analysis. HPAHs: heavy polycyclic aromatic hydrocarbons; PCBs: polychlorobiphenyls; PBDEs: polybromodimethylethers; DDTs: dichlorodiphenyltrichloroethanes, dichlorodiphenyldichloroethanes and dichlorodiphenyldichloroethylenes; SOD: superoxide dismutase; GPx: glutathione peroxidase; MDA: malondialdehyde; G: gills; DG: digestive gland; M: mantle; P: plasma; B: Bouin; BOY: Boyard; LP: Les Palles; ML: Mus du Loup; C: Cordouan; s: summer; w: winter; 1: replicate 1; 2: replicate 2; 3: replicate 3.

results could be explained by the fact that PAHs can lead to the formation of ROS and enhance oxidative stress in aquatic organisms (Di Giulio et al., 1989; Winston, 1991). Indeed, different studies have shown an increase in SOD activity in bivalves that have been exposed to hydrocarbons (Orbea et al., 2002; Richardson et al., 2008; Sole et al., 1995), suggesting that hydrocarbons induce oxidative stress by producing ROS such as O_2^-. A significant correlation between PAH accumulation in the flesh and SOD and CAT activities in the gills has been found in mussels from the Mediterranean Sea (Porte et al., 1991). Sole et al. (1995) also reported correlations between GPx activity in the digestive gland of molluscs collected on the Mediterranean coast and their organochlorine body burden and SOD activity in the digestive gland as well as PAH concentrations. Krishnakumar et al. (1995) reported higher CAT activity in the digestive gland of mussels from contaminated sites when compared to those sampled at a reference site in the North American Pacific coast. Consistently, increased antioxidant activities were found in bivalves from contaminated sites in terms of PCBs, PAHs and pesticides (De Luca-Abbott et al., 2005; Fernandez et al., 2010). In the present study, the results also suggest that HPAH and/or DDT contamination increase the MDA levels in the gills and digestive gland.

SOD activities in the digestive gland correlated negatively with the second RDA axis (Fig. 4a). These results suggest that PCB, PBDE and/or lindane contamination decrease the SOD activity in the digestive gland. This could be attributed to the inhibition of the enzyme synthesis by these contaminants or to enzyme inactivation by high contaminant tissue concentrations, as has been suggested for other contaminants (Borg and Schaich, 1983).

Laccase in the plasma and mantle correlated positively with the second RDA axis (Fig. 4a). These results suggest that PCB, PBDE and/or lindane contamination increase laccase activities in plasma (Fig. 4a). Thus, laccase activity may enable protection against bioaccumulated PCBs, PBDEs and lindane, and therefore, may be a potential defence biomarker candidate in ecotoxicological studies, as suggested in previous reports (Luna-Acosta et al., 2011a,b). However, there is a lack of fundamental understanding about the activation and regulation of key factors of catecholase-type and laccase-type PO pathways in bivalves. Thus, more studies on the potential roles of these enzymes in oysters are needed for a better understanding of the potential implications of their modulations in the presence of contaminants.

Results of RDA analysis suggest that PCB, PBDE and/or lindane contamination can increase lysozyme activity in the digestive gland (Fig. 4a), suggesting that lysozyme plays a role in the detoxification of PCB, PBDE and/or lindane in *C. gigas*.

Proteins in the digestive gland correlated negatively with the second RDA axis (Fig. 4a). This result suggests a negative correlation between the protein content in the digestive gland and PCB, PBDE and lindane levels, and therefore, are in agreement with the hypothesis that contaminants may exert an effect on oyster energy reserves, that is, exposure to contaminants induces a lower accumulation of energy reserves, or energy reserves are deployed in the presence of these contaminants for the proper activation of defence mechanisms. However, no correlations were observed between lipid and glycogen levels in the digestive gland and contaminant body burdens. Therefore, more studies are needed to better assess the relationship between contaminant contents and energy reserves in bivalves.

HPAHs and DDTs strongly correlated with the first RDA axis, and the condition index correlated negatively with the first axis (Fig. 4a). This suggests negative correlations between the condition index and HPAH and DDT content, suggesting that these contaminants may exert a negative effect on the oyster condition index. However, the clear North–South gradient effect on condition index values in summer (Fig. 3c) suggests that other internal or external factors could have an effect on the condition index.

3.3. Potential effects of internal and external factors on juvenile oyster biomarker responses

Possible effects on biomarker responses could be attributed to internal factors such as oyster body size or gross body state (Packard and Boardman, 1999), which are estimated in the present study through flesh dw, shell dw and the condition index. MANCOVA analyses revealed no significant effect of the tested covariates on the biomarker responses, suggesting that the oyster body size or gross body state had no effect on the biomarker responses.

Possible effects on biomarker responses could also be attributed to external environmental factors such as temperature. Data collected in 2000–2006 and in 2008, from French monitoring networks in the same area of the present study (Table 3), suggest that differences in some environmental parameters between sites follow a North–South gradient. A slight increase in temperature was observed in a North–South gradient in summer, higher levels of turbidity were observed in winter, especially in B, BOY and LP, and differences in phytoplankton abundance and species composition were observed between sites and seasons, with a higher abundance of phytoplankton and species composition observed in summer, especially in C (Table 3). However, no clear modulations for the biomarker responses were observed in a North–South gradient in the present study, with the exception of the condition index (Fig. 3c). It is important to mention that the condition index varies, principally, with the availability and/or the quality of the

food (Mourgaud et al., 2002). Therefore, the results of the present study suggest that the trophic quality in C is better than in the other sites and that, in the present study, the condition index is influenced more by the seawater temperature or the presence and/or quality of food than by contaminants. Amongst other environmental variables (salinity, presence of phycotoxins, faecal contamination), no particular differences were observed between the sites and seasons (Table 3).

Overall, with the exception of the condition index, these results suggest that environmental factors such as temperature, salinity, turbidity, phytoplankton abundance and species composition, phycotoxins and faecal contamination were not the main causes of variation in biomarker responses between the sites and seasons. However, further studies should be carried out in order to verify this hypothesis.

3.4. Use of transplanted juvenile oysters to differentiate sites depending on their contamination levels and biological effects

Site sample analysis (Fig. 4b) shows three well-defined groups, depending on the levels of contaminants. Group 1 is clearly defined by the levels of PCBs, PBDEs and lindane, group 2 is clearly defined by the levels of HPAHs and DDTs, and group 3 encompasses all the other sites, that is, BOYs, MLs, Cs and Cw. Similar correlations were observed between contaminant levels and MDA in the gills and digestive gland and between contaminant levels and SOD in the plasma and mantle (Fig. 4a), suggesting that, if only two tissues should be selected for in situ studies in *C. gigas*, only the measurement of MDA in the digestive gland and the measurement of SOD in plasma would be enough to discriminate sites with high levels of HPAH and DDT contamination. In addition, GPx in plasma could contribute towards discriminating sites with high levels of HPAH and DDT contamination. On the other hand, SOD and lysozyme in the digestive gland and laccase in plasma could contribute towards the discrimination of sites with high levels of PCB, PBDE and lindane contamination. Thus, in the present study, SOD, GPx, MDA, laccase, lysozyme and proteins in the digestive gland as well as SOD, GPx and laccase in plasma contributed to discriminate sites depending on their degree of contamination.

In a transplantation study carried out in Ebro Delta (NE Spain) with freshwater clams (*Corbicula fluminea*) over a three-month period, the measured biomarker responses (antioxidant and esterase enzyme responses as well as lipid peroxidation) showed marked differences across the sites and the months, depending on the levels of pesticides present at these sites (Damasio et al., 2010). In a transplantation study in the Cecine River (Italy), the freshwater painters mussel (*Unio pictorum*) was transplanted for four weeks (one month) in different locations of the river basin, and the bioaccumulation of metals was integrated with a wide battery of biomarkers consisting of oxidative, genotoxic and lysosomal responses (Guidi et al., 2010). Considering the mild contamination gradient in the investigated area, the overall results suggested that some oxidative biomarkers, as well as those evaluating chromosomal and cell damage, were highly sensitive and could be profitably applied to caged painters mussels for environmental quality assessment in freshwater. In a transplantation study on the South coast of Portugal, transplanted mussels *Mytilus galloprovincialis* exhibited significant alterations in some biomarkers, which reflected the type of contaminants present at each site (metals or PAHs), and the biomarkers that differentiate the impact of different contaminants at each site were metallothioneins, cytochrome P450, SOD and CAT measured in the digestive gland. Thus, the results of the present study are in agreement with previous in situ studies, in which biomarker responses have shown marked differences across sites, depending on the level of contamination.

4. Conclusion

In conclusion, amongst the battery of biomarkers applied to the present work (including antioxidant defence mechanisms, humoral immune defence mechanisms and physiological biomarkers), MDA and lysozyme in the digestive gland as well as SOD, GPx and laccase in plasma contributed to significantly discriminate between the sites in which oysters bioaccumulated different levels of HPAHs, DDTs, PCBs, PBDEs and lindane. In combination with other recent studies, these results strengthen the hypothesis that it is possible to differentiate sites depending on their contamination levels and biological effects by carrying out studies with transplanted juvenile oysters.

Interestingly, the SOD, GPx and MDA levels positively correlated to HPAH and DDT body burdens and were associated with different sites in the Marennes–Oléron Bay (LP, BOY and ML). These results suggest potential oxidative stress, leading to lipid peroxidation in juvenile oysters, and seem to be related to local inputs of HPAHs and DDTs, probably from the Charente and the Seudre rivers, and not to external inputs from the Gironde Estuary. An increase in oxidative stress and lipid peroxidation could affect defence mechanisms in juvenile oysters, which may contribute towards the increase in disease and mortality of *C. gigas* observed in the Marennes–Oléron Bay. Therefore, more studies should be performed in order to further examine the effects of these organic contaminants on *C. gigas*.

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