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## **Levels and Distribution pattern of PCB congeners in *Ensis siliqua* (Linnaeus, 1758) from the Islas Cies, Galicia (N.W., Spain) in relation to biometric parameters**

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**Levels and Distribution pattern of PCB congeners in *Ensis siliqua* (Linnaeus, 1758) from the Islas Cies, Galicia (N.W., Spain) in relation to biometric parameters**

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Manuscripts

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3 **Levels and distribution pattern of PCB congeners in razor clams *Ensis***  
4 ***siliqua* (Linnaeus, 1758) from Galicia (N.W., Spain) in relation to**  
5 **biometric parameters**  
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27 To establish polychlorinated biphenyl (PCB) levels and distribution as well as their  
28 relationship to biometric parameters in the razor clam *Ensis siliqua*, 15 individual  
29 samples collected from two points sited in the Islas Cies, Ría de Vigo (Galicia, Spain),  
30 collected monthly in the period from February 2003 to April 2004, were analysed. PCBs  
31 (IUPAC nº 31, 28, 52, 101, 118, 153, 105, 138, 156 and 180) were isolated by Soxhlet  
32 extraction. The identification and quantification were performed by GC/MS and GC  
33 (electron capture detector, ECD), respectively. The isomer concentrations in the razor  
34 clam *E. siliqua* were in the order hexachlorobiphenyls > pentachlorobiphenyls >  
35 heptachlorobiphenyls > trichlorobiphenyls > tetrachlorobiphenyls. No correlation was  
36 observed between the *E. siliqua* shell length and PCBs levels. The PCBs 31, 101, 105  
37 and 153 levels were statistically related with some biometric parameters (p<0.05).  
38 Multivariate techniques such as Principal Component Analysis (PCA) show clear  
39 seasonal differences between samples.  
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51  
52 **Keywords:** PCBs, Biometric parameters, Gas chromatography, *Ensis siliqua*, Galician  
53 coast, PCA (Principal component Analysis).  
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## Introduction

Galicia in north-west Spain is an important shellfish producing region, characterized by a peculiar geography with a succession of estuarine bay systems called Rías which give good oceanographic conditions for aquaculture, mainly for farming bivalve molluscs. The razor clam *Ensis siliqua* (Linnaeus, 1758) is a benthic bivalve organism that lives in the bottom of sandy sediments in the infralittoral region. It is found in the middle and external zones in the Ría de Vigo (Rolán 1989). The Islas Cies belong to the National Park of Atlantic Islands, they are three islands sited in the mouth of the Ría de Vigo.

Polychlorinated biphenyls (PCBs) are biologically, chemically and physically stable in the environment. They are hydrophobic and persistent contaminants, which bioaccumulate along the food chain (Erickson 1997). The fat content and condition index of mollusk are strongly connected to the reproductive cycle because a part of their lipid content is lost during the spawning period. Moreover, the gonad cycle is closely related to environmental conditions and seasons (Hawkins and Bayne 1992; Brousseau 1995). So, PCBs are generally distributed in marine organisms as a consequence of condition index and lipid content variations, mainly during reproduction periods.

There is a lack of information relating to hydrophobic baseline micropollutant levels in *E. siliqua* from the Galician coast. Few regional studies about reproductive cycle and biometric parameters of this species in Galicia coast have been reported (Darriba et al. 2005). The objective of this work was to study the levels and distribution of 10 PCBs, recommended by the International Council for Exploration of the Seas (ICES) (Duinker et al. 1988) with IUPAC number 31, 28, 52, 101, 118, 153, 105, 138, 156 and 180 in *E.*

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2  
3 *siliqua* collected monthly from two points of The Islas Cíes, Ría de Vigo (Playa de Aras  
4 das Rodas and Playa de San Martin) during the period from February 2003 to April  
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8 2004. Univariate and multivariate techniques were applied in order to check the  
9  
10 influence of biometric parameters on PCBs levels and to establish any the spatial and  
11  
12 temporal trends.  
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## 17 **Materials and Methods**

### 19 *Sampling. Biometric parameters*

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21  
22 Samples of 15 adults specimens of sizes between 120 and 160 mm coming from two  
23  
24 points of The Islas Cíes (Ría de Vigo), Playa de Aras das Rodas and Playa de San  
25  
26 Martin, were monthly collected during the period from February 2003 to April 2004  
27  
28 (See Figure 1). Each specimen was measured and the flesh was dissected from the  
29  
30 shells. Condition indices were calculated taking account shell and body weights of each  
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32 sample.  $CI = (\text{body weight} / (\text{body} + \text{shell weights}))$ .  
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36 [Insert Figure 1 about here]  
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### 41 *Chemicals and CRM*

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3 N-pentane, dichloromethane and isooctane for organic trace analysis and aluminium  
4 oxide (alumina), silica gel and anhydrous sodium sulfate were purchased from Merck  
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6  
7  
8 (Darmstadt, Germany). Analytical reagent grade PCB individual congener standards  
9  
10 were supplied by Dr. Ehrenstorfer (Augsburg, Germany). A certified reference material,  
11  
12 mussel tissues homogenate, purchased from National Institute of Standards and  
13  
14 Technology (NIST 2977) was used for quality control.  
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17

#### 18 19 20 *Standard solutions and calibration*

21  
22 Solutions of Standard stock (crystalline solid) were prepared by weighing a appropriate  
23  
24 amount of each standard and diluting to 5 mL with isooctane. Working solutions were  
25  
26 made by dilution of the solution of stock. For quantitative gas chromatographic  
27  
28 determinations, calibration was performed at five concentration levels for each congener  
29  
30 spanning the range of 1-400 ng g<sup>-1</sup> and using CB 155 (500 ng g<sup>-1</sup>) as internal standard.  
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33

#### 34 35 36 *Sample preparation*

37  
38 Samples were homogenized. Homogenates of the flesh were frozen (-30 °C), freeze-  
39  
40 dried and Soxhlet extracted (5 g of sample; 150 mL of dichloromethane-pentane, 1-1; 8  
41  
42 hours) by duplicate. The sample extract was concentrated under vacuum evaporation to  
43  
44 dryness and then redissolved in 1 mL of isooctane. An aliquot of the extract was used to  
45  
46 determine gravimetrically the lipid content. Interfering lipids were removed from a  
47  
48 suitable portion of the concentrate by column chromatography on alumina (6%  
49  
50 deactivated). The sample extract was eluted with n-pentane. The fraction of PCBs was  
51  
52 isolated using column chromatography on Silica (1% deactivated) and eluted with  
53  
54 isooctane. CB 155 was added as an internal standard prior to analysis by gas  
55  
56 chromatography (González-Quijano and Fumega 1996).  
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### *Chromatographic analysis*

Sample extracts (1  $\mu\text{L}$ ) were analyzed individually by separate runs by high resolution GC using a Perkin-Elmer Autosystem GC with an electron capture detector (ECD). A TRB-5 (Teknokroma, Spain) 5 % diphenyldimethyl siloxane capillary column (60 m x 0,20 mm i.d. x 0,4  $\mu\text{m}$  phase thickness) was used. The chromatographic conditions were: the column temperature program was 90° C (3 min.) to 215 °C (40 min.) at 30 °C/min. and 275°C (30 min.) at 5°C/min.; the injector temperature (splitless mode, 1,8 min.) was 270 °C; the ECD temperature was 365 °C; carrier gas was hydrogen purchased from Air Liquid (Spain). The confirmation of polychlorinated biphenyl compounds was performed by injecting a 1  $\mu\text{L}$  of sample extract in a Varian Saturn 2000 Gas Chromatograph-Ion Trap Detector Mass Spectrometer (ITD). The experimental conditions and capillary column were identical to those described before; carrier gas was helium (Air Liquid, Spain). The mass conditions were: the trap temperature 170 °C; the scan range 100-450 m/z; the multiplier delay 6 min.; the emission current 10  $\mu\text{A}$ ; the multiplier voltage 1600 V; the maximum ionization time 25000  $\mu\text{s}$ . Data numerical analysis was performed by means of the Minitab 14 statistical package.

### *Quality control*

The QA/QC (Quality Control/ Quality Assurance) procedures included analysis of blanks, duplicates and Standard Reference Materials (SRMs). Curves of calibration have been carried out at five concentration levels using suitably diluted standards (working solutions). Each concentration level has been injected in duplicate and peak heights have been fitted by linear regression. The coefficient of correlation was 0,9999

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3 for all the target compounds. The linearity range was from 3 to 300 ng g<sup>-1</sup> for all  
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6 studied compounds.  
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10 Method reproducibility studies were performed for single compounds on five replicate  
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12 samples (mixtures of PCBs), analyzed by two people on different days. The mean  
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14 relative standard deviation ranged from 8 to 10 % for the PCBs which were studied.  
15  
16 The percentage recovery of PCBs were investigated by an exhaustive Soxhlet extraction  
17  
18 of *E. Siliqua* spiked by slowly pouring it over acetone containing the target compounds  
19  
20 (PCBs 31, 28, 52, 101, 118, 153, 105, 138, 156 and 180). The sample was manually  
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22 mixed for 30 min and allowed to air-dry for 7 days and stored in the dark for a month  
23  
24 before analysis. The expected final concentrations were calculated to range from 15 to  
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29 20 ng.g<sup>-1</sup>. The mean recoveries of target compounds were from 80.0 to 101.2 %.  
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34 A study of method accuracy was performed on a certified reference material, mussel  
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36 tissues homogenate, NIST 2977. The PCBs content agreed with the certified value. The  
37  
38 certified content for the organochlorine compounds, PCBs 31, 28, 52, 101, 118, 153,  
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41 105, 138, 156 and 180 are  $3.92 \pm 0.24$ ,  $5.37 \pm 0.44$ ,  $8.37 \pm 0.54$ ,  $11.2 \pm 1.2$ ,  $10.5 \pm 1.0$ ,  
42  
43  $14.1 \pm 1.0$ ,  $3.76 \pm 0.49$ ,  $16.6 \pm 1.6$ ,  $0.96 \pm 0.09$  and  $6.79 \pm 0.67$  ng g<sup>-1</sup>, respectively.  
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48 Detection limits were calculated as three times the standard deviation of the peak height  
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50 for 30 determinations of the blank and were for PCBs 31, 28, 52, 101, 118, 153, 105,  
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52 138, 156 and 180, 0.04, 0.1, 0.08, 0.08, 0.1, 0.06, 0.07, 0.04, 0.04 and 0.01 ng L<sup>-1</sup>,  
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54 respectively. The limits of quantification were calculated as 10 times the standard  
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56 deviation of the peak height for 30 determinations of the blank and were for PCBs 31,  
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3 28, 52, 101, 118, 153, 105, 138, 156 and 180, 0.13, 0.32, 0.28, 0.26, 0.33, 0.21, 0.22,  
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5 0.13, 0.14 and 0.04 ng L<sup>-1</sup>, respectively.  
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## 10 **Results and discussion**

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12 The average of two analytical replicates of ten individual PCB congener concentrations  
13 (IUPAC number 31, 28, 52, 101, 118, 153, 105, 138, 156 and 180) expressed in ng g<sup>-1</sup>  
14 dry weight (dw) of *E. siliqua* coming from two studied points and monthly collected in  
15 the period from February 2003 to April 2004 is shown in the Table I. The ΣPCBs is the  
16 sum of all individual PCB congeners. The levels of ΣPCBs varied in the range of 2.75 to  
17 67.70 ng g<sup>-1</sup>. *E. siliqua* collected in the playa de San Martin in February 2003 and in the  
18 playa de A. Rodas in January 2004, the winter months, gave the highest concentrations  
19 of ΣPCBs. The lowest value appeared in July 2003 in playa de San Martin. This  
20 behaviour could be due to unfavourable meteorological conditions in winter which  
21 coincide with extremely high rain fall (162 mm. of total precipitation in January and  
22 February 2003 and 106 mm. of total precipitation in January 2004, Anuario  
23 climatológico de Galicia 2003 y 2004) and rates of re-suspension attributed to storm  
24 events that can cause inputs of high PCBs levels. In summer, however, there was  
25 hardly any inflow contributions and for this reason the PCBs were found in samples at  
26 very low levels (40 mm. of total precipitation in July 2003, Anuario climatológico de  
27 Galicia 2003). This situation recurs constantly in Galician coast and in other littorals  
28 and ecosystems (Carro et al. 2004; Olafsdottir et al. 1998). Table I shows that samples  
29 collected in April 2003 were less contaminated than samples collected in April 2004.  
30 The two sampling points had very similar concentrations in this month.  
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[Insert Table I about here]

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6 The isomer concentrations of *E. siliqua* body were in the order  
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8 hexachlorobiphenyls>pentachlorobiphenyls>heptachlorobiphenyls>trichlorobiphenyls>  
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10 tetrachlorobiphenyls. Hexachlorobiphenyls 153 and 138 were the most prevalent  
11  
12 congeners accounting for 47.6% and 19.2% of the total PCBs levels, respectively. This  
13  
14 indicates that the biota is exposed predominantly to higher chlorinated PCB  
15  
16 formulations. PCBs 153 and 138 are the main constituents in Arochlor 1254 and 1260  
17  
18 because they were the most largely used in commercial mixtures in European countries  
19  
20 (Ivanov and Sandell 1992). On the other hand, both PCBs belong to the group of  
21  
22 difficult to metabolize congeners. These percentages are consistent with the studies of  
23  
24 PCBs in other marine organisms (Carro et al. 2004; Storelli and Marcotrigiano 2003).  
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32 In Figure 2 it can be seen that *E. siliqua* coming from playa de San Martin had higher  
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34 levels of hexachlorobiphenyl congeners, less mobile and less water soluble compounds,  
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36 than those coming from playa de A. Rodas. This means that playa de San Martin point  
37  
38 is nearer a source of contamination. Low chlorinated biphenyls, the most water soluble  
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40 compounds, were predominant in biota coming from playa de A. Rodas, point away  
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42 from a source. This hypothesis is supported by the fact that the mean of  $\Sigma$ PCBs in *E.*  
43  
44 *siliqua* coming from playa de San Martin (13.24 ng g<sup>-1</sup>) was slightly higher than the  
45  
46 mean of  $\Sigma$ PCBs in samples coming from playa de A. Rodas (9.72 ng g<sup>-1</sup>). However, this  
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48 conclusion cannot be conclusively confirmed because the differences between zones  
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50 were not statistically significant (see statistical analysis).  
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58 [Insert Figure 2 about here]  
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3 The percentage lipid content was found at the lowest levels during February-April,  
4 when the only period of spawning occurred (see the Table I). The average lipid content  
5 was similar for samples coming from the two studied points, 5.50% for playa de San  
6 Martin and 5.64% for playa de A. Rodas.  
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15 Biota coming from playa de San Martin possessed slightly worse biometric  
16 characteristic, shell length, 133 mm and condition index, 0.56, compared to *E. siliqua*  
17 from playa de A. Rodas, 136 mm. and 0.59, respectively, it is logical and consistent  
18 with PCBs levels. As previously mentioned, the point in playa de San Martin underwent  
19 slight conditions of environmental stress. The two studied zones are geographically very  
20 close, so biometric parameters were similar.  
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### 32 *Statistical analysis.*

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34 The statistical analysis considered concentrations of 10 PCBs and 3 biometric  
35 parameters as quantitative variables. Levels reported as “Not detected” were assigned of  
36 half the detection limit. All data were tested for homogeneity of variance (Kolmogorov  
37 Smirnov Test) and analyzed using Analysis of Variance (One Way ANOVA. Fisher’s  
38 method) in order to check the influence of biometric parameters on chemical variables.  
39  
40 This analysis indicated a significant effect ( $p < 0.05$ ) of quantitative biometric  
41 parameters, fat content on PCB 101 levels and condition index on PCBs 31 and 153  
42 levels. The relationship of PCB congeners levels to lipid content is very complex, fat  
43 nature, extraction methods and difference between individual organisms have an  
44 important role which is difficult to know (Ewald et al. 1998). Moreover, the levels of  
45 PCBs are very low and the analysis methods are not able to pick up the small  
46 differences among individuals collected monthly, induced by the variation in lipid.  
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6 No statistical significance was observed between shell length and concentration of  
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8 PCBs. So, the accumulation of pollutants by *E. siliqua* organism could have reached a  
9  
10 steady state and their elimination rate, mainly caused by their reproductive cycle, would  
11  
12 be sufficiently fast to compensate for the dietary intake.  
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16  
17 Spatial and temporal trends were also studied using one-way ANOVA. If the variable  
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19 month was grouped in seasons: spring, summer, autumn and winter, only fat content  
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21 and PCB 105 levels showed a significant season term using the ANOVA ( $p=0.00$  and  
22  
23  $p=0.031$ , respectively). PCBs 101 and 180 levels were statistically influenced by the  
24  
25 sampling months ( $p=0.00$ ). No statistical significance was found for sampling zones,  
26  
27 which is logical because both points are in the same estuarine bay, Ría de Vigo, and  
28  
29 they have been subjected to similar environmental conditions, exhibiting a similar  
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31 biological cycle and therefore a similar trend in biometric parameters and not very  
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33 different PCBs levels.  
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41 The significant differences between seasons and levels of PCBs and biometric  
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43 parameters were supported by PCA that provided an easier visualization of the  
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45 relationship among variables in a large data set. PCA is a multivariate technique that  
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47 tries to reduce the number of original variables explaining most of variance in the data,  
48  
49 it transforms a set of correlated measured variables into a set of a few not correlated  
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51 components. If PCA was applied to original data, the retained first three principal  
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53 components explained the 79.6 % of total data set. Table II shows the loading of the  
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55 variables in the first three components of the PCA, that is to say the contribution of the  
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57 original variables to each principal component. The first component had high negative  
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3 loading for higher chlorinated biphenyls (PCBs 101, 118, 138, 156, 180 and  $\Sigma$ PCBs),  
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5 the second component had high negative loading for trichlorobiphenyl compounds  
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7 (PCBs 31 and 28) and PCB 105 and positive loading for fat content. The third  
8  
9 component had high scores for shell length, condition index and PCB 52 (negative  
10  
11 ones) and PCB 153 (positive one). In the analysis of the Pearson correlation  
12  
13 coefficients, the higher chlorinated biphenyls had a strong correlation between them, the  
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15 same behavior presented the lower chlorinated biphenyls, it could be due to either the  
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17 probable similar profile of input and bioaccumulation or the same origin of both kinds  
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19 of compounds.  
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27 [Insert Table II about here].  
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32 In Figure 3a it can be seen the PCA scores plot, distribution profiles for the studied  
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34 samples. In the plane of the first two components, three groups corresponding to the  
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36 samples collected in spring, summer and autumn appear.  
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41 The group of the samples collected in winter (January, February and March) had a great  
42  
43 dispersion. The most polluted sample (the highest value of  $\Sigma$ PCBs), collected in  
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45 February 2003 in playa de San Martin, was defined by negative scores on PC1 and PC2,  
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47 they had a high concentration of the lower and higher chlorinated biphenyls at the same  
48  
49 time. The sample collected in March 2003 in playa de A. Rodas also showed an high  
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51 negative score for PC2, this sample had the highest levels for the trichlorobiphenyls,  
52  
53 PCBs 31 and 28, and for the PCB 105 (see Table II). Samples collected in January 2004  
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55 showed high negative scores on PC1, having high concentrations of the higher  
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57 chlorinated compounds (PCBs 101, 118, 138, 156, 180 and  $\Sigma$ PCBs).  
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6 [Insert Figure 3a about here]  
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10 The first group, formed by samples collected in spring (April, May and June), showed  
11 different degree of negative scores on PC2, these samples were contaminated by the  
12 lower chlorinated biphenyls and PCB105 in more or less degree depending on the  
13 month (May or April), samples from May were situated nearer from the origin of  
14 coordinates, then were less contaminated. Samples collected in April 2004 were  
15 associated with the negative part of PC1 and to the positive part of PC2, showing a  
16 greater contamination with the higher chlorinated compounds and a higher fat content.  
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29 The second and first group, samples collected in summer and autumn had positive  
30 scores for PC1 and PC2. Summer samples were distributed along PC1, those collected  
31 in September were defined by the highest positive scores on PC1 and PC2. These  
32 samples were the least contaminated and contained the highest fat amounts, period  
33 before spawning.  
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43 All samples collected in 2004 were related to the negative part of the PC1 and the  
44 positive part of PC2, having high levels of PCBs 101, 118, 138, 156 and 180 and  
45  $\Sigma$ PCBs.  
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53 In Figure 3b it can be seen that the samples of the two zones which were studied (playa  
54 de San Martin and A. Rodas) were strongly associated with the sampling month, and no  
55 spatial and temporal trends were observed, it shows that the contamination by PCBs  
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3 was irregular over the studied sampling months and shows no upward or downward  
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5 trend.  
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10 [Insert Figure 3b about here]  
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## 15 **Conclusions**

16  
17 Due to the low statistical significance between biometric parameters and levels of these  
18 hydrophobic pollutants, it is not necessary to take into account the lipid content or  
19 condition index when data are expressed. It is very useful in monitoring programs  
20 because biometric parameters are influenced by other complex factors such as  
21 reproductive cycle and physiological state (internal factors) and/or meteorological and  
22 environmental conditions (external factors) and/or even, analytically speaking, their  
23 determination or measurement. This benthic organism can be used as bioindicator in  
24 zones where mussels are not cultured because they are able to bioaccumulate  
25 contaminants to much higher concentrations than those found in water and to the extent  
26 of showing clear seasonal differences in level of PCBs. Another important reason for  
27 considering the razor clam as a good bioindicator is that the distribution patterns of PCB  
28 congeners are similar to those of other marine organisms such as mussel. This work is a  
29 good tool for establishing the background levels of PCBs in *E.siliqua* from the Galicia  
30 coast.  
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## 60 **References**

1  
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Table I. Average concentration of PCBs ( $\text{ng g}^{-1}$ ) in *E. siliqua* from two points in The Islas Cies (R. Vigo). Biometric parameters: Shell length (mm), fat (%), condition index.

<b>San Martin</b>													
Month	Length	FAT	CI	$\Sigma$ PCB	CB31	CB28	CB52	CB101	CB118	CB153	CB105	CB138	CB156
Feb.03	133.93	2.96	0.62	67.7	0.77	0.27	N.D.	0.81	0.88	61.59	0.82	2.34	N.D.
March 03	132.33	3.17	0.5888	3.27	N.D.	0.14	N.D.	0.27	N.D.	2.1	N.D.	0.76	N.D.
April 03	134.73	2.93	0.6233	6.17	1.92	0.93	0.14	0.32	0.2	1.23	0.09	1.02	0.21
May-03	128.13	5.84	0.5113	5.73	0.66	0.36	N.D.	0.67	0.43	2.06	0.15	1.3	0.1
July 03	134.33	6.59	0.6255	2.75	0.07	0.04	0.13	0.51	0.48	N.D.	0.22	1.25	N.D.
Aug.03	133.53	7.14	0.6315	5.34	0.11	N.D.	0.14	0.59	0.57	2.04	0.31	1.51	N.D.
Sept.03	138.53	8.05	0.1892	4.7	0.16	0.14	0.04	0.65	0.47	1.76	N.D.	1.43	N.D.
Oct.03	120.27	3.54	0.6455	8.85	0.05	0.14	0.23	0.79	0.18	2.29	N.D.	2.76	0.22
Nov.03	136.87	7.03	0.5849	5.3	0.33	0.11	0.11	0.59	0.39	1.71	N.D.	1.56	N.D.
Dec.03	137.26	8.3	0.6189	4.31	0.24	0.1	0.09	0.64	0.48	N.D.	N.D.	2.05	N.D.
Jan.04	141	4.43	0.5485	26.48	0.21	0.19	0.46	3.37	1.06	12.51	0.33	0.18	0.66
April 04	129	6.09	0.6299	18.34	0.07	0.08	0.18	1.9	0.68	7.79	0.2	6.18	0.54
<b>Aras das Rodas</b>													
Month	Length	FAT	CI	$\Sigma$ PCB	CB31	CB28	CB52	CB101	CB118	CB153	CB105	CB138	CB156
Feb.03	133.73	2.84	0.6591	5.54	0.71	0.34	0.78	0.32	N.D.	2.35	N.D.	1.04	N.D.
March 03	143.4	3.95	0.5965	9.04	1.64	1.4	0.06	0.58	0.37	2.12	0.87	1.64	0.32
April 03	137.87	2.74	0.5782	6.53	0.84	0.35	N.D.	0.52	0.44	2.12	0.61	1.55	0.1
May-03	128.67	4.42	0.615	5.93	0.68	0.74	N.D.	0.6	0.46	2.03	0.08	1.34	N.D.
July 03	136.73	6.7	0.6351	3.86	0.09	N.D.	0.11	0.43	0.46	1.56	0.01	1.14	0.01
Aug.03	133.27	6.87	0.6394	4.4	0.86	0.27	0.16	0.4	0.35	1.35	0.05	0.94	N.D.
Sept.03	128.6	7.56	0.2213	3.82	0.1	0.09	0.08	0.35	0.43	1.42	0.12	1.15	0.02
Oct.03	132.23	6.77	0.6088	5.73	0.12	0.03	0.22	0.57	0.66	1.45	N.D.	1.9	N.D.
Nov.03	144.21	7.9	0.6384	4.7	0.07	0.09	0.22	0.55	0.39	1.17	N.D.	1.45	N.D.
Dec. 03	136.13	6.52	0.638	11.29	0.52	0.16	0.08	0.74	0.45	2.93	N.D.	3.21	0.25
Jan.04	138.67	5.5	0.5941	36.6	0.27	0.15	0.54	4.55	1.23	16.27	0.44	2.84	1.01
April 04	140.1	6	0.6218	19.18	0.08	0.06	0.2	1.97	0.7	8.03	0.16	6.43	0.43

N.D. Not detected

Table I. Average concentration of PCBs ( $\text{ng g}^{-1}$ ) in *E. siliqua* from two points in The Islas Cies (R. Vigo). Biometric parameters: Shell length (mm), fat (%), condition index.

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4	
5	<u>CB180</u>
6	0.22
7	N.D.
8	0.11
9	N.D.
10	0.05
11	0.07
12	0.05
13	2.19
14	0.5
15	0.71
16	7.51
17	<u>0.68</u>
18	
19	<u>CB180</u>
20	N.D.
21	0.04
22	N.D.
23	N.D.
24	0.05
25	0.02
26	0.06
27	0.78
28	0.76
29	2.95
30	9.3
31	<u>1.12</u>

N.D. Not detected

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Table II. Loading of the variables in the first three components of the PCA

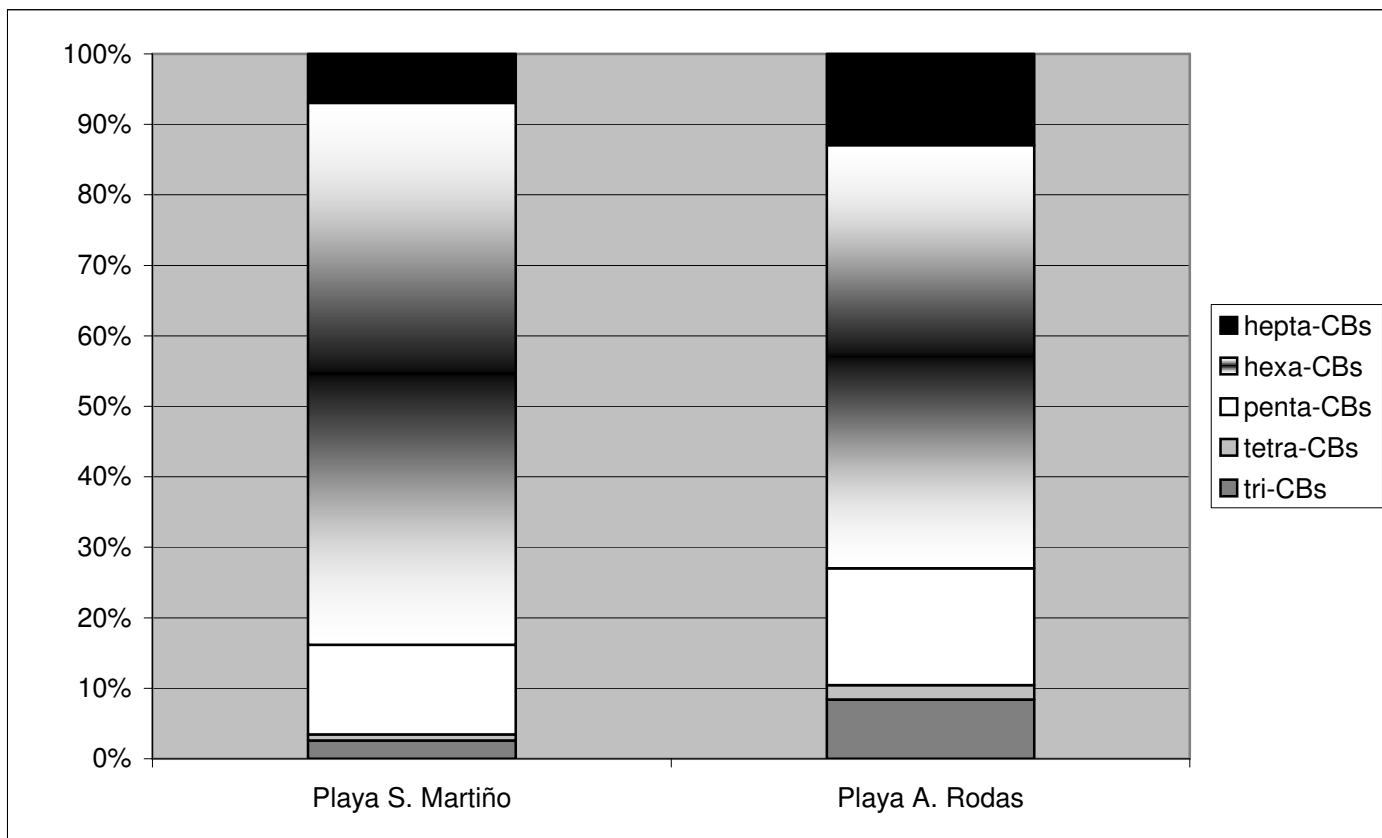
Variable	PC1	PC2	PC3
$\Sigma$ CB	-0,365	-0,159	0,356
Length	-0,135	-0,054	-0,162
FAT	0,086	0,423	0,126
IC	-0,071	-0,134	-0,176
CB31	0,032	-0,508	-0,205
CB28	0,032	-0,491	-0,253
CB52	-0,199	0,119	-0,448
CB101	-0,422	0,144	-0,171
CB118	-0,396	0,113	0,166
CB153	-0,273	-0,204	0,477
CB105	-0,238	-0,381	0,188
CB138	-0,173	0,131	0,169
CB156	-0,393	0,045	-0,271
CB180	-0,379	0,147	-0,268

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Map with sampling points.  
177x125mm (300 x 300 DPI)

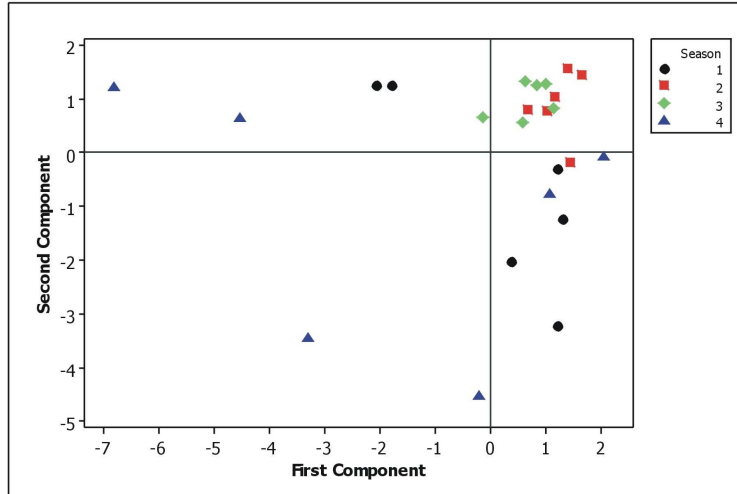
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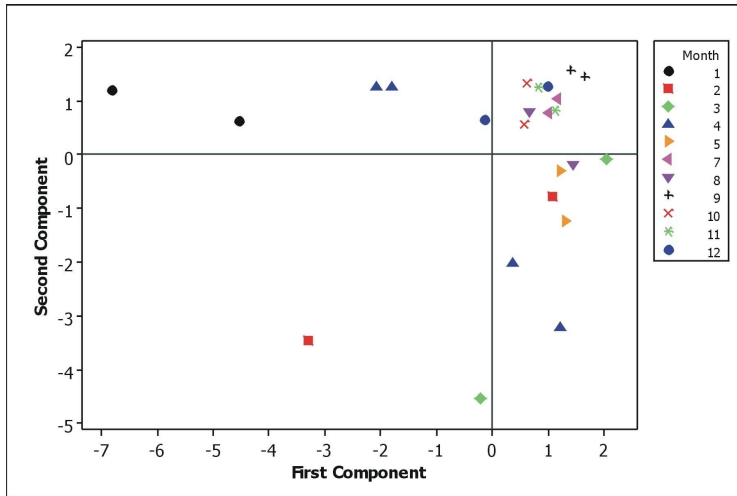
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a. Scores plot. Distribution of *Ensis siliqua* samples collected in the different seasons in the plane of the first and second components. 1. Spring (April, May, June), 2. Summer (July, August, September), 3. Autumn (October, November, December), 4. Winter (January, February, March).  
 b. Scores plot. Distribution of *Ensis siliqua* samples collected in the different months in the plane of the first and second components. 1. January, 2. February, 3. March, 4. April, 5. May, 6. June, 7. July, 8. August, 9. September, 10. October, 11. November, 12. December.

158x246mm (300 x 300 DPI)