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Organochlorine Pesticide Levels in *Ensis siliqua* (Linnaeus, 1758) from Ría de Vigo, Galicia (N.W. Spain): Influence of Season, Condition Index and Lipid Content

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Abstract Levels of organochlorine pesticides (OCPs), including ΣDDTs, γ-HCH, HCB, aldrin, isodrin, *trans*-nonachlor, heptachlor and dieldrin, were determined in the razor clam, *Ensis siliqua,* collected monthly from February 2003 to April 2004 from the Islas Cíes in Ría de Vigo (Galicia, Spain). The sum of DDTs ranged from 2.17 to 26.9 ng g^{-1} dry weight (dw). Principal component analysis showed seasonal trends in the levels of some OCPs (γ-HCH and dieldrin). Pearson correlations ($p < 0.05$) were observed between OCP levels and the biometric parameters of condition index and body lipids.

Keywords OCPs · Biometric parameters · *Ensis siliqua* · Galician coast

The widespread use of organochlorine pesticides, OCPs, in agriculture has led to important environmental emissions over the last decades. Their high persistence, toxicity and tendency to bioaccumulate have resulted in use bans for some OCPs in developed countries since the 1970s. However, many developing countries still use OCPs for agricultural purposes because of their high efficiency and low cost.

Marine micropollution represents a potential risk to seafood production, mainly to benthic organisms such as bivalve molluscs. Razor shells of the genus *Ensis* are the most economically important species of razor clams in the European Union (Freire et al. 2008). The razor clams are typical feeder bivalves that live buried in the sandy sediment in the infralittoral region. In Galicia (North West Spain), *Ensis siliqua* 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, and consist of three islands located in the mouth of the Ría de Vigo.

The use of bivalve molluscs, mainly mussel and oyster, for monitoring programs of OCP profiles in littoral seawater is widely accepted and recommended by international conventions (OSPAR 2000). Mussels concentrate pollutants to levels above those present in marine water (Kennish 1997) and are considered to be indicators of chemical contaminants in a specific area. There is little information about the use of *E. siliqua* as a bioindicator of pollution. This species is not as extensively cultivated as the mussel, *Mytilus galloprovincialis*. In general, quantitative data on OCP levels in marine organisms for the Galician coast are scarce (Alvarez-Piñeiro et al. 1995; Carro et al. 2004).

The objective of this work was to study the levels and distribution of OCPs in *E. siliqua* collected monthly at two points of The Islas Cíes, Ría de Vigo (Playa de Aras das Rodas and Playa de San Martiño) during the period from February 2003 to April 2004, and to thereby contribute to the characterization of the Galician coast with respect to contamination by OCPs. Univariate and multivariate statistical techniques were applied to evaluate the influence of biometric parameters on OCP levels, and to determine if any seasonal trends were evident.

Materials and Methods

The coordinate ranges (GPS, WGS84) of sample collection sites were 42°12′11″ N and 8°54′5″ W for San Martiño and

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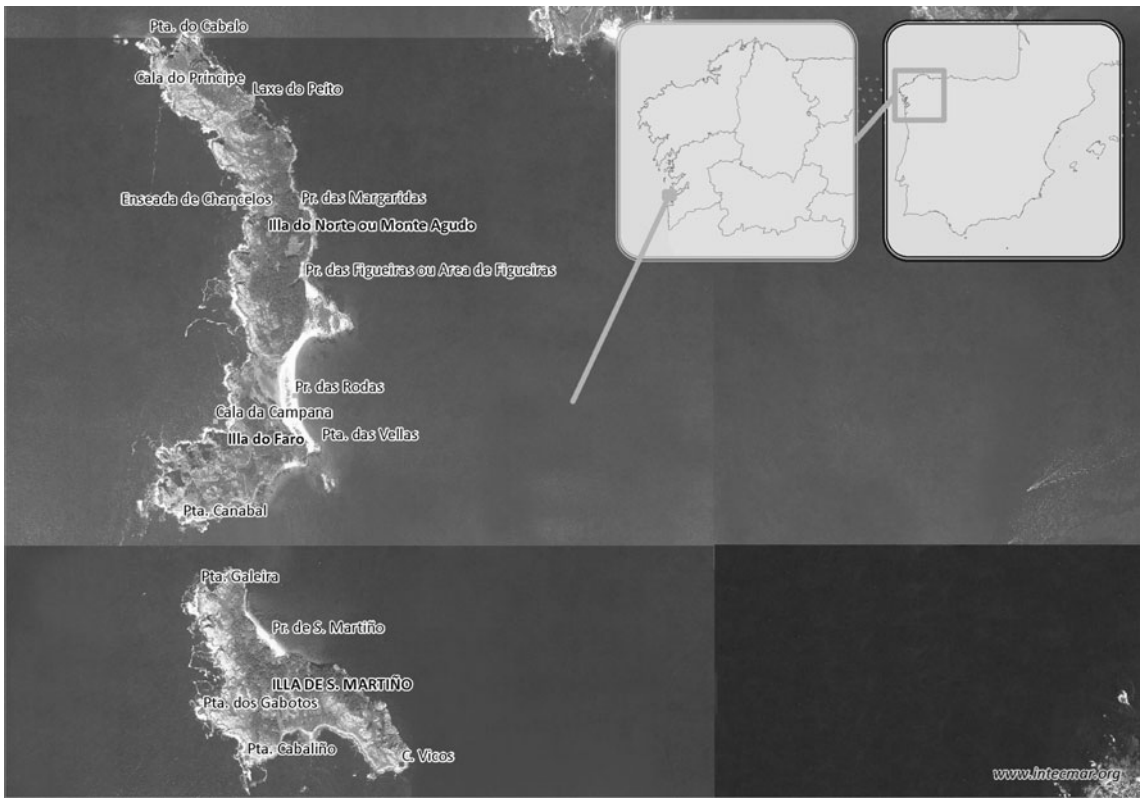

Fig. 1 Map with sampling points

42°13′24″ N and 8°53′54″ W for Rodas. Samples were collected monthly during the period from February 2003 to April 2004 (Fig. 1) and stored at −20°C until analysis. Each specimen was measured and the flesh was dissected from the shells. Condition indices were calculated taking into account shell and body weights of each sample. Lipid content was gravimetrically determined.

Individual standards of op′-DDT, pp′-DDT, pp′-DDD, pp′-DDE, γ-HCH, HCB, aldrin, isodrin, *trans*-nonachlor, heptachlor and dieldrin were supplied by Dr. Ehrenstorfer (Augsburg, Germany) and Supelco (Bellefonte, PA, USA). N-pentane, dichloromethane and isooctane for trace organic analysis, anhydrous sodium sulfate, alumina and silica for column chromatography were purchased from Merck (Darmstadt, Germany). Standard stock solutions were made by weighing a suitable amount of each organochlorine pesticide standard and diluting to 5 mL with isooctane. Working solutions were prepared by dilution of the stock solution. Certified reference materials, mussel tissue homogenates, were purchased from the National Institute of Standards and Technology (NIST 2977 and NIST 1974b), and used for quality control.

Fifteen adult specimens of sizes between 120 and 160 mm were used for each analysis. Homogenates of the flesh were frozen (−30°C), freeze-dried and Soxhlet extracted (5 g of sample; 150 mL of dichloromethane-pentane, 1–1; 8 h) in duplicate, and the sample extract was concentrated under vacuum evaporation to 1 mL. An aliquot of the extract was used to determine the lipid content. Lipids were removed from a suitable portion of the concentrate by column chromatography on alumina (6% deactivated). The fraction of organochlorine pesticides was isolated using column chromatography on silica (1% deactivated) (González-Quijano and Fumega 1996).

The concentrated extracts were analyzed by high resolution gas chromatography using a Perkin-Elmer autosystem gas chromatograph with an electron capture detector (ECD) (Perkin Elmer, Norwalk, CT, USA). The confirmation of pesticides was performed on a Varian Saturn 2000 gas chromatograph-ion trap detector mass spectrometer (ITD) (Varian Inc., CA, USA).

Numerical analysis of the data was performed by means of the Minitab 15 statistical package (Manugistic, Inc., Rockville, MD, USA). Pearson product–moment correlation coefficient is a measure of the lineal relationship between two variables. One way analysis of variance (ANOVA) is used to know if there are statistically significance differences between variables. Principal components analysis (exploratory technique) provides few linear combinations (loadings) of original variables reducing the initial set and extracting a low number of factors (components), in this way the relationship between the variables and samples classification are observed.

Results and Discussion

The detection limits were calculated as three times the standard deviation of the peak height for 30 determinations of the blank, and were from 0.02 to 0.1 ng g^{-1}. Method accuracy was evaluated using certified reference materials (mussel tissue homogenates NIST 2977 and NIST 1974b). The assigned levels for individual OCPs ranged from 1.3 to 41.0 ng g^{-1} dry weight (dw). The mean recoveries of OCPs spiked into tissue samples ranged from 63% to 109%, and the reproducibility of analytical method in relative standard deviations ranged between 4% and 26%. The laboratory participated biannually in the quality assurance of information for marine environmental monitoring in Europe (QUASIMEME laboratory performance studies, LPS), involving intercomparison exercises for some pesticides in biota.

The residue levels of OCPs and biometric parameters (length, lipid content and condition index) in samples collected from San Martiño and Rodas points are presented in Table 1. The results presented are consistent with data reported by several authors in other molluscs (Solé et al. 1994; Licata et al. 2004). The sum of DDTs ranged from 2.17 to 26.9 ng g^{-1} dw. The concentration ranges of γ-HCH, HCB, aldrin, isodrin, *trans*-nonachlor, heptachlor and dieldrin were below the level of detection (LOD) in some samples.

It is evident from Table 1 that the residues of ΣDDTs (sum of op′- and pp′-DDT and its metabolites, pp′-DDD and pp′-DDE), γ-HCH (lindane), HCB, aldrin, isodrin, *trans*-nonachlor, heptachlor and dieldrin, were present in varying amounts in samples from the two studied points. ΣDDTs was the most abundant pollutant followed by the cyclodienes (*trans*-nonachlor and isodrin). HCB, an important by-product in PCB synthesis, was determined in very low levels due to reduced use and its lower bioaccumulation factor (Saito et al. 1992). The levels of γ-HCH in all samples were of the order of sub-ppb and even below

Table 1 Concentration expressed in ng g^{-1} dry weight for the studied OCPs and biometric parameters (length in mm, lipid content in % and condition index) in *Ensis siliqua* coming from Islas Cies in Ría de Vigo (Galicia, Spain) during the period February 2003 to April 2004

Zone/month	Length	Lipid content	Condition index	ΣDDT	γ-HCH	HCB	Aldrin	Isodrin	*Trans*-nonaclor	Heptaclor	Dieldrin
Martiño/Feb03	133.9	3.0	0.62	16.6	0.73	0.26	0.29	0.70	1.16	2.31	ND
Martiño/Mar03	132.3	3.1	0.59	4.36	0.26	0.17	ND	ND	0.69	0.67	ND
Martiño/Apr03	134.7	2.9	0.62	21.9	0.78	0.24	ND	1.05	1.40	0.13	ND
Martiño/May03	128.1	5.8	0.51	17.5	0.76	0.40	0.26	1.24	2.88	0.07	ND
Martiño/Jul03	134.3	6.6	0.63	7.91	0.65	0.36	0.47	1.24	1.65	0.25	0.91
Martiño/Aug03	133.5	7.1	0.63	9.98	0.91	0.69	1.75	0.32	ND	0.90	0.59
Martiño/Sep03	138.5	8.1	0.19	2.17	0.48	0.05	0.20	0.71	ND	0.07	0.51
Martiño/Oct03	120.3	3.5	0.65	7.74	ND	ND	ND	0.87	1.08	ND	ND
Martiño/Nov03	136.9	7.0	0.58	4.41	ND	0.07	ND	0.32	1.54	ND	0.24
Martiño/Dec03	137.3	8.3	0.62	4.75	ND	0.08	ND	1.06	1.43	ND	0.19
Martiño/Jan04	141.0	4.4	0.55	2.41	0.58	0.12	0.42	0.28	0.47	0.82	0.29
Martiño/Apr04	129.0	6.1	0.63	4.46	0.45	0.09	0.35	ND	0.62	0.57	0.25
Rodas/Feb03	133.7	2.8	0.66	7.49	0.43	0.30	0.55	0.42	1.28	0.21	ND
Rodas/Mar03	143.4	3.9	0.60	10.5	0.56	0.62	ND	0.36	1.24	ND	ND
Rodas/Apr03	137.9	2.7	0.58	20.4	0.81	0.30	ND	1.33	1.62	0.89	1.22
Rodas/May03	128.7	4.4	0.62	13.7	0.56	0.33	ND	0.84	2.48	0.04	ND
Rodas/Jul03	136.7	6.7	0.64	7.25	0.60	0.31	ND	0.73	1.22	ND	1.86
Rodas/Aug03	133.3	6.9	0.64	5.69	0.74	0.06	0.01	1.70	1.61	0.44	1.29
Rodas/Sep03	128.6	7.6	0.22	3.64	0.97	0.11	1.24	2.06	0.79	0.12	0.32
Rodas/Oct03	132.2	6.8	0.61	7.14	ND	0.10	ND	0.83	0.67	ND	0.21
Rodas/Nov03	144.2	7.9	0.64	5.17	ND	0.08	ND	0.35	1.48	3.14	ND
Rodas/Dec03	136.1	6.5	0.64	26.9	ND	0.07	0.10	0.39	1.29	ND	0.11
Rodas/Jan04	138.7	5.5	0.59	3.67	0.66	0.12	0.30	0.22	0.36	0.77	0.31
Rodas/Apr04	140.1	6.0	0.62	3.59	0.49	0.12	0.52	0.26	ND	0.85	0.44

ND: below the LOD

the LOD, even though our samples were collected in an area close to the Ría de Vigo, Polígono de Torneiros (Porriño, Pontevedra, Spain), where the soil has been declared as contaminated by γ-HCH (Official Journal of Galicia no. 136 July 13, 2000). These findings are in line with the results obtained by other authors. Although γ-HCH accumulates in food chains, it has a lower half-life in animal tissues than other OCPs. γ-HCH does not appear to pose a problem for marine systems (Kennish 1997), as levels found in shellfish samples coming from mussel watch projects were around the detection limit.

Differences between the two sampling points were not relevant. However samples from Rodas in the northern island presented slightly higher levels of OCPs than samples from San Martiño. This finding is contrary to a study of contamination by PCBs performed in the same region (Carro et al. 2006), where it was shown that Rodas had the lowest levels of indicator PCBs. Both PCBs and OCPs have a similar chemical nature. They are hydrophobic and lipophilic compounds that tend to bioaccumulate in lipids, and are considered as indicators of anthropogenic contamination. A similar accumulation mechanism may be considered in these two contaminant families.

In general, the highest levels of each compound were found between February 2003 and August 2003 for samples from San Martiño, and between March 2003 and September 2003 for samples from Rodas (see Table 1). It is interpretable with the intensive use of pesticides during the spring and the summer seasons. The minor pesticides, mainly cyclodienes, presented very sparse values of concentration. This made temporal trends difficult to observe. In relation to major compounds, the sum of DDTs presented the highest levels in April 2003 for San Mariño samples and in December 2003 for Rodas samples. The spring and autumn months presented a highly unstable weather pattern, which coincided with extremely heavy rainfall. This could have caused inputs of high OCP levels. The sum of DDTs presented the lowest values in summer, September 2003, when inflow contributions were negligible.

Statistical analysis considered 8 organochlorine pesticides and 3 biometric parameters as quantitative variables; concentrations of contaminants reported as "below the detection limit" were assigned a value of one-half the detection limit. Sampling year, month and season were considered as qualitative variables.

Pearson product-moment correlations (Minitab 15) were used to determine if there was a relationship between levels of organochlorine pesticides and biometric conditions (length, lipid content and condition index) in investigated samples. Coefficients suggested the existence of slightly significant correlation ($p < 0.05$) between isodrin and heptachlor levels and shell length ($p = 0.03$ and 0.045, respectively), between HCB level and the lipid content ($p = 0.016$) and, between isodrin level and condition index ($p = 0.019$), only for Rodas samples (samples with slightly higher levels of OCPs). But unlike the PCBs measured in this species (Carro et al. 2006), the relationship was negative for the OCPs. This finding is not logical given the lipophilicity of these compounds. However, it is consistent with the results of Carro et al. (2004), where cyclodiene pesticides (isodrin and endrin) presented a slight negative correlation with fat in mussels. Larsson et al. (1991) also observed a negative relationship between certain other micropollutant concentrations and tissue lipid content in eels. In general, when the concentrations of micropollutant are low (trace compounds), errors associated with their determination may distort the interpretation of data analysis. One hypothesis that has been advanced for this negative relationship is that the pollutants are diluted in the increasing fat amounts of biota providing an inverse relationship between OCPs concentration and lipid content (Ferrante et al. 2010).

All data were analyzed using ANOVA (One Way ANOVA, Fisher's method) to evaluate possible relationships between OCP levels and temporality. If the variable month was grouped in seasons: spring, summer, autumn and winter, the analysis indicated a significant effect of season on γ-HCH and dieldrin levels ($p = 0.00$, 0.005, respectively). Lipid content was statistically influenced by the sampling months ($p = 0.00$) for γ-HCH, *trans*-nonachlor and dieldrin levels ($p = 0.001$, 0.04 and 0.026, respectively). Analyzing data from each sampling location separately showed that γ-HCH and dieldrin had a

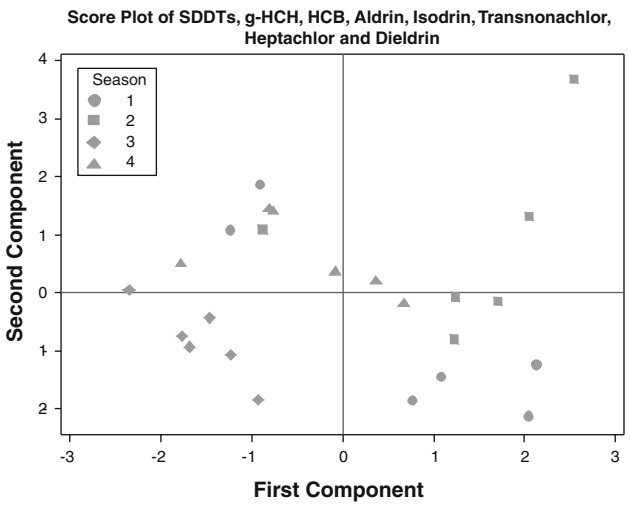

Fig. 2 Scores plot. Distribution of *Ensis siliqua* samples collected in the different seasons in the plane of the first and second components. *1* Spring (April, May, June), *2* Summer (July, August, September), *3* Autumn (October, November, December), *4* Winter (January, February, March)

significant seasonal term in ANOVA for San Martiño samples and γ-HCH for Rodas samples.

The significant relations between seasons and levels of OCPs were supported by PCA, a multivariate statistical test. The first three principal components explained 68.5% of the total variation. The PCA plot is shown in Fig. 2. In the plane of the first two components, four groups corresponding to the samples collected in spring, summer, autumn and winter appeared.

The majority of samples collected in the spring were related to the positive part of PC1 and the negative part of PC2. Increasingly positive PC1 scores along this axis indicate increasing concentrations of γ-HCH, HCB and isodrin and increasingly negative PC2 scores along this axis describe increasing ΣDDTs and *trans*-nonachlor levels in *E. siliqua*. Two samples collected in April 2004 in San Martiño and Rodas were related to the negative and positive regions of PC1 and PC2, respectively corresponding to significant aldrin levels.

Except for one sample from San Martiño collected in September 2003, summer samples were distributed along the positive axis of PC1, with increasingly positive PC1 scores characterized by increasing levels of γ-HCH, HCB and isodrin in *E. siliqua*. Half of them were distributed along the positive part of PC2 (samples from San Martiño and Rodas collected in August and September 2003 with high concentrations of aldrin) and the other half along the negative part of PC2 (samples from San Martiño collected in July 2003 and from Rodas collected in July and August 2003 with relatively high concentrations of ΣDDTs and *trans*-nonachlor).

The autumn samples were related to the negative aspects of PC1 and PC2. Increasingly negative scores here were characterized by increasing amounts of ΣDDTs and *trans*-nonachlor levels in *E. siliqua* samples.

Samples collected in the winter months were distributed along the positive region of PC2 (high level of aldrin), except for the sample from Rodas collected in March 2003. This sample was related to the negative part (high level of ΣDDTs and *trans*-nonachlor). Most of these samples were related to the negative region of PC1 (Samples from San Martiño collected in March 2003 and in January 2004, and from Rodas collected in February 2003 and in January 2004, with low concentrations of the other pesticides).

In summary, samples collected in spring were characterized by high levels of ΣDDTs and *trans*-nonachlor and low concentration of aldrin, except for two samples collected in 2004 that had high levels of the latter. In spring and summer there were samples with increased concentrations of commonly used insecticides or fungicides, γ-HCH and HCB, possibly derived from their intensive use. Both pesticides are strongly associated and Pearson correlated ($p = 0.008$). This may be due to the fact that HCB

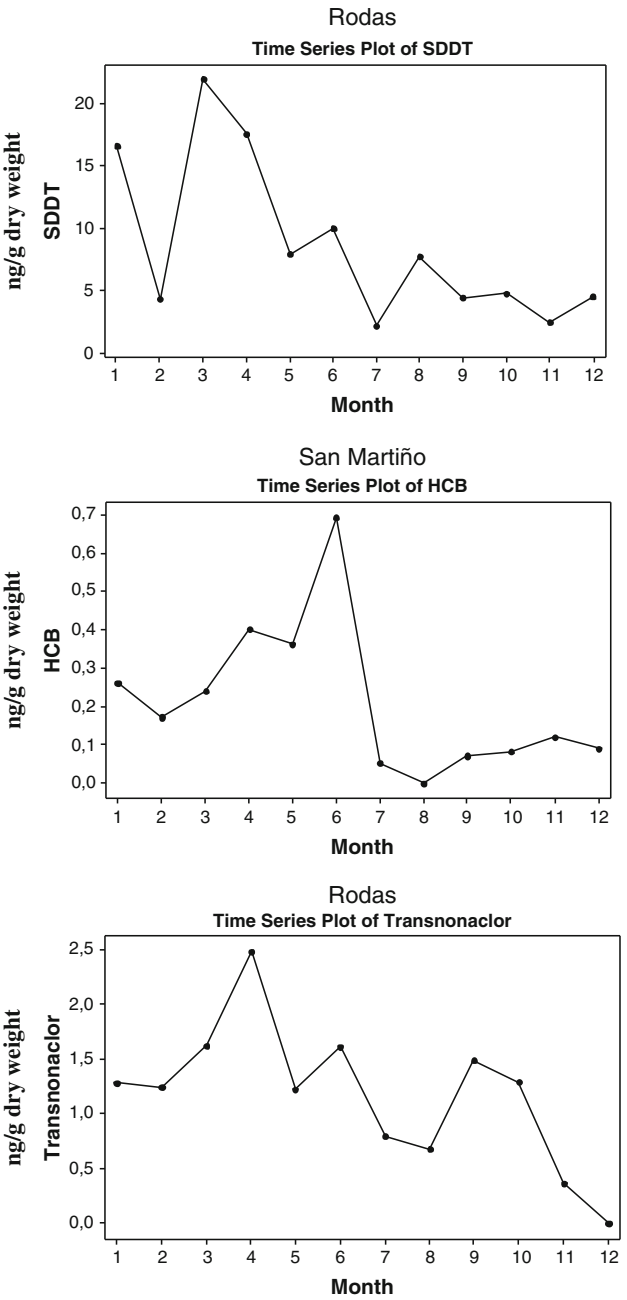

Fig. 3 Time series plot of ΣDDTs and transnonachlor levels in *Ensis siliqua* coming from Rodas and of HCB levels from San Martiño, respectively. The *x* axis represents the months of sampling (during the period from February 2003 to April 2004)

is known to be a residue in γ-HCH applications. Samples collected in autumn had the same pattern as samples collected in the spring. In winter, the contamination level was low overall, with the exception of aldrin in 2004. In general, the studied OCPs had higher concentrations in *E. siliqua* during the spring and summer months than the autumn and winter months.

Although OCPs are ubiquitously distributed in the environment and cause a background contamination, the

results shown above demonstrate a slight seasonality in *E. siliqua*. This may be due to the seasonal use of these compounds in agricultural activities in the immediate region. However, a portion may also be derived from long-distance transport, as observed by Bizzotto et al. (2009). The samples collected in our study were just offshore from a deserted island complex (Islas Cíes), where there is no or very limited use of OCPs.

Samples in this study were collected from February 2003 (Fig. 3, month 1) to April 2004 (Fig. 3, month 12). Samples were not collected in June of 2003 or in February and March of 2004. Figure 3 shows a shallow decline in OCP concentrations over this time period for the main compounds present, namely ΣDDTs and *trans*-nonachlor at Rodas and HCB at San Martiño. A decline of OCPs levels in aquatic media (biota and sediment) has been observed in recent years due to the substitution of OCPs for less persistent pesticides (Hendriks and Pieters 1993; Solé et al. 1994; Covaci et al. 2005; Vorkamp et al. 2008).

Further investigations are needed to determine if biometric data for *E. siliqua* may be useful in OCP monitoring programs with this species. It is concluded from this study that *E. siliqua* can be used as a bioindicator species where more commonly tested mussel species do not exist.

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