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Concentrations of Contaminants with Regulatory Limits in Samples of Clam (*Chamelea gallina*) Collected along the Abruzzi Region Coast in Central Italy

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ABSTRACT

Concentrations of pollutants with regulatory limits were determined in specimens of *Chamelea gallina,* a species of clam collected along the Abruzzi coastal region of the central Adriatic Sea. Nine sampling sites were selected to evaluate the distribution of contaminants in the environment and the health risk for consumers. The concentrations of all the examined compounds were lower than the maximums set by European legislation. Polycyclic aromatic hydrocarbons and total mercury were below the detection limit (0.18 μg/kg for benzo[*a*]anthracene, 0.30 μg/kg for chrysene, 0.12 μg/kg for benzo[*b*]fluoranthene, 0.08 μg/kg for benzo[*a*]pyrene, and 0.0050 mg/kg for total mercury) in all the analyzed samples. Mean concentrations of lead and cadmium were 0.104 and 0.110 mg/kg, respectively. Of the non–dioxin-like polychlorinated biphenyls, PCB-153, PCB-180, and PCB-138 were the most abundant at all sampling sites (1a to 9a) at 0.25 mi (ca. 0.4 km) and at some sampling sites (1b, 2b, 3b, 5b and 7b) at 0.35 mi (ca. 0.56 km). Principal component analysis revealed that the concentrations of dioxin-like polychlorinated biphenyls were similar at the majority of sampling sites, and O8CDD and 2,3,7,8-T4CDF were the predominant dioxin congeners.

The clam *Chamelea gallina* (Linnaeus 1758) is a filter feeding organism widespread along the eastern Atlantic coasts from Norway to the British Islands, Portugal, Morocco, and the Canary Islands. Bivalve mollusk fishing is an important economic activity along the Italian coasts of the Adriatic Sea, and *Chamelea gallina* is a commercial species particularly popular in restaurants and home-cooked meals. During the 1980s, fishing activity for this clam increased greatly after the introduction of hydraulic dredges, but in recent years a population decrease has been observed due to several irregular mortality phenomena *(28)*. These could be the result of various events, such as changes in environmental factors (high temperature or reduced salinity), stress conditions during the reproductive period, aquaculture practices, pathogens, and pollutants *(1, 25)*. Chemical contaminants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and heavy metals can increase the expression of heat shock proteins, which play an important role in responses to several stress factors *(27)*. Toxic anthropogenic compounds can impact the immune system of bivalve mollusks, altering the functional activity of hemocytes involved in phagocytosis *(24)*. In marine ecosystems, many contaminants bind organic suspended particles and then precipitate onto sediments, where the contaminants can accumulate in high concentrations. Therefore, polluted sediments represent a potential risk for benthic organisms, such as bivalves, in which the speed of absorption is greater than that of metabolic transformation and elimination. Bivalve mollusks can be used for pollution assessment studies and monitoring programs because they are sessile organisms during most of their life cycle, have a world wide geographical distribution, and concentrate pollutants to higher concentrations than those present in marine water by filtering large volumes of water and removing suspended particles of 1 to 250 μm *(20)*.

Because of their filter feeding and slow rate of detoxification, mollusks can accumulate many toxic contaminants. Among the heavy metals, lead (Pb), cadmium (Cd), and mercury (Hg) are toxic, and maximum concentrations have been set by legislation in various foodstuffs intended for human consumption *(38)*. The toxic effects of lead affect the developing nervous system and the hematological and cardiovascular systems. Cadmium is classified as a human carcinogen *(13)*; kidney and bone are the targets of its toxicity following oral exposure *(32)*. Mercury is one of the most hazardous environmental pollutants because of its adverse neurological effects on adults but especially on fetuses, neonates, and young children *(31)*.

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TABLE 1. *Maximum concentrations of chemical contaminants allowed according to European legislation*

Compound	Maximum concn	European Commission regulation no.
Benzo[*a*]pyrene	5.0 μg/kg	835/2011 *(10)*
Sum of benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, and chrysene	30.0 μg/kg	835/2011 *(10)*
Sum of PCDDs and PCDFs (WHO-TEQ)[a]	3.5 pg/g	1259/2011 *(11)*
Sum of PCDDs, PCDFs, and DL-PCBs (WHO-TEQ)	6.5 pg/g	1259/2011 *(11)*
Sum of NDL-PCBs (PCB28, PCB52, PCB101, PCB138, PCB153, and PCB180)	75.0 ng/g	1259/2011 *(11)*
Lead	1.5 mg/kg	1881/2006 *(8)*
Cadmium	1.0 mg/kg	1881/2006 *(8)*
Mercury	0.5 mg/kg	1881/2006 *(8)*

[a] WHO-TEQ, toxic equivalents according to the World Health Organization.

Other pollutants such as PCBs, characterized by lipophilicity, stability, and a persistence in the environment, tend to accumulate up the food chain and pose potential hazards to any marine organisms or humans consuming them. These pollutants can cause chloracne, skin discoloration, liver dysfunction, reproductive effects, dermatitis, dizziness, developmental problems, and cancer *(6)*. Several PCBs have toxicological effects similar to those of dioxins (polychlorinated dibenzo-*p*-dioxins [PCDDs] and dibenzofurans [PCDFs]) and thus are called dioxin-like PCBs (DL-PCBs). Technical PCB mixtures used in toxicity studies contain both non–DL-PCBs (NDL-PCBs) and DL-PCBs, but simultaneous exposure to these compounds does not allow an estimation of their toxicity or toxic concentrations alone. These mixtures exert a variety of toxicological effects on liver, thyroid, immune function, reproduction, behavior, and carcinogenicity *(12, 18)*. PCDDs and PCDFs, commonly referred to as dioxins, are organochlorine compounds belonging to a list originally comprising 12 persistent organic pollutants. The toxicity of individual dioxin congeners differs considerably. Among 210 congeners, only 17 have significant toxicity because of the replacement of hydrogen with chlorine atoms, at least in the 2,3,7,8-positions *(35, 36)*. The responses include dermal toxicity, immunotoxicity, carcinogenicity, and adverse effects on reproduction, embryo development, and endocrine function *(5, 16–18, 21, 33, 34)*.

Regarding PAH toxicity, numerous studies indicate that the noncarcinogenic compounds produce oxidative stress and affect the immune system, endocrine regulation, and development, whereas high-molecular-weight PAHs are carcinogenic *(37)*. Taking into account the toxicity of all these classes of pollutants, the European Union set maximum allowable concentrations (Table 1) for lead, cadmium, mercury, PAHs, PCDDs, PCDFs, DL-PCBs, and NDL-PCBs in many foodstuffs, including mollusks *(8, 10, 11),* and prescribed monitoring programs in the EU member states. Directive EC No 56/2008 *(9)* established a framework within which member states should take the necessary measures to maintain a clean and healthy marine environment by the year 2020 at the latest. According to Annex I of this Directive, contaminants in fish and other seafood for human consumption must not exceed concentrations established by EU legislation (*8, 10, 11*) or other relevant standards. The aim of the present study was to investigate the presence of pollutants, e.g., heavy metals, PAHs, PCDDs, PCDFs, DL-PCBs, and NDL-PCBs in *C. gallina*. Concentrations in samples of *C. gallina* collected along the Adriatic coast were compared with regulatory limits to assess the environmental status of this marine subregion of the Mediterranean Sea and to evaluate safety of this mollusk for human consumption.

MATERIALS AND METHODS

Sampling. This survey was carried out in collaboration with fishing cooperatives during the winter (December 2013 through February 2014) because the contaminant concentrations are likely higher in winter than in spring or summer. The spawning period of *C. gallina* is long, running from April to October, and during this period the concentrations of lipophilic contaminants can decrease. The sampling area involved the Abruzzi region (province of Chieti) along the central Adriatic Sea coast (Fig. 1). The coordinates for the sampling sites from which the clams were collected are listed in Table 2. Clams were collected from nine sites (1a to 9a) at 0.25 mi (ca. 0.4 km) but only five sites (1b to 3b, 5b, and 7b) at 0.35 mi (ca. 0.56 km) because of lack of specimens. All clams were commercial size (25 mm) and were harvested by hydraulic dredge. Clams were kept in boxes with ice until arrival at the laboratory within 3 h. From each of the sampling sites, 250 clams were shelled and homogenized to obtain a pool for specific analyses.

All analyses were conducted in duplicate, and the results are reported as the mean in all the tables.

The analytical methods followed those of the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise G. Caporale as the Italian National Reference Laboratory for PCDDs, PCDFs, DL-PCBs, and NDL-PCBs.

Determination of PAHs: reagents and standards. The reference standard PAH mix 240 contained benzo[*b*]fluoranthene, benzo[*a*]anthracene, benzo[*a*]pyrene, and chrysene, all at 1 μg/ml in toluene (Dr. Ehrenstorfer-Schafers Laboratories, Augsburg, Germany). Five calibration solutions were prepared evaporating 10, 20, 50, 100, and 200 μl of PAH mix 240 in five vials and then reconstituting with 1 ml of acetonitrile, the same organic solvent as used in mobile phase. The final concentrations of PAHs were 10, 20, 50, 100, and 200 ng/ml. The reference material QPH072BT, a commercially available mussels tissue spiked with PAHs (Quasimeme, Wageningen, The Netherlands), contains benzo[*b*]fluoranthene (3.90 ± 0.59 μg/kg), benzo[*a*]anthracene (3.10 ± 0.49 μg/kg), benzo[*a*]pyrene (1.77 ± 0.32 μg/kg), and chrysene (4.27 ± 0.63 μg/kg). The solvents acetonitrile, ethyl acetate, hexane, and dichloromethane (Sigma-Aldrich, St. Louis, MO) were organic residue analysis quality. The salts used were Original QuEChERS (1 g NaCl and 4 g $MgSO_4$; Agilent, Santa Clara, CA), and the

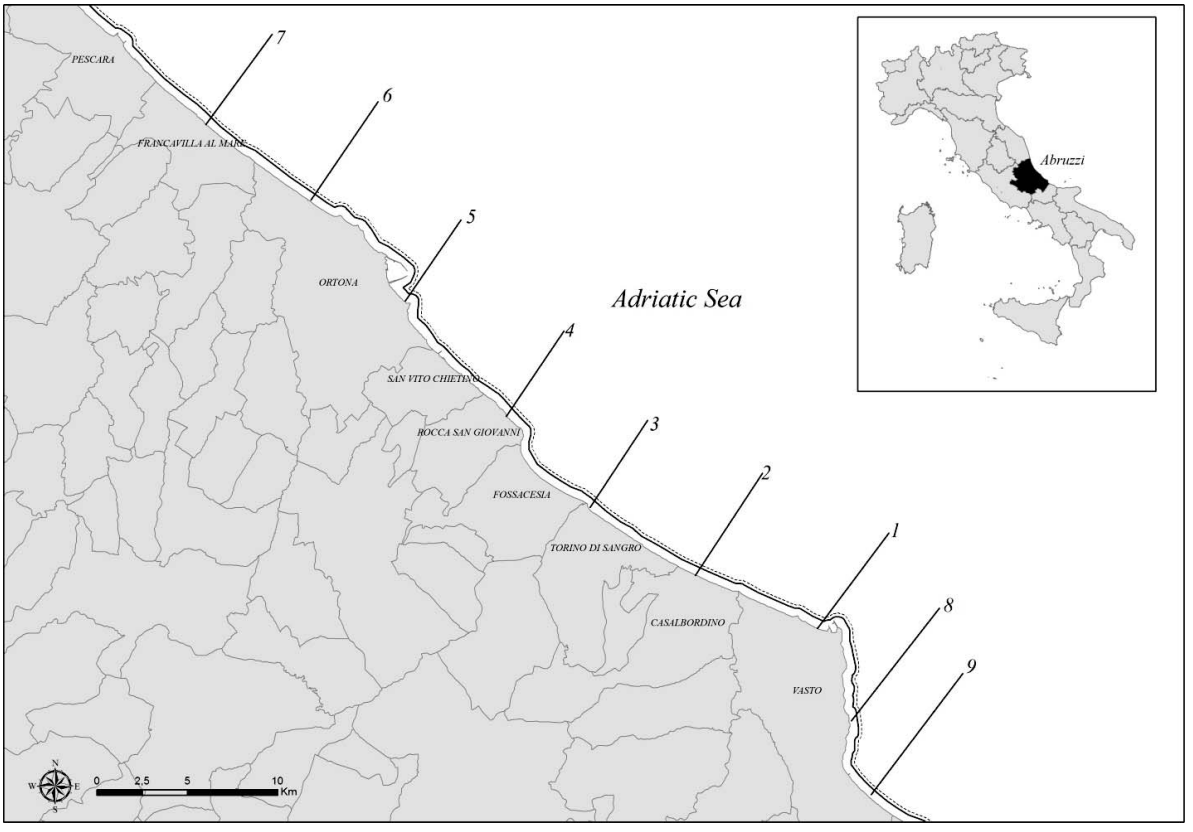


FIGURE 1. *Sampling sites for* Chamelea gallina *along the central Adriatic Sea coast.*

cartridge was Strata EPH silica based (200 μm, 70 Å, 5 g/20 ml; Giga Tubes Teflon, Phenomenex, Torrance, CA).

Determination of PAHs: sample preparation. A 50-ml falcon tube was used to mix 10 g of the homogenized sample, 5 ml of water (just enough to optimize the extraction step and minimize the formation of emulsion), and 10 ml of ethyl acetate at 180 rpm for 15 min. The Original QuEChERS salts were added, and the tube was shaken manually for 1 min. After centrifugation at 4,500 rpm for 5 min at 4°C, 5 ml of supernatant was transferred into a 7-ml glass vial and evaporated under a nitrogen stream not exceeding 40°C. The remainder was taken up with 1 ml of hexane. The clean up was performed with the Strata EPH silica-based cartridge, conditioned with 10 ml of hexane-dichloromethane (3:1, vol/vol) and 8 ml of hexane. After the sample was loaded, the analytes were eluted with 15 ml of hexane-dichloromethane (3:1, vol/vol), and the eluate was evaporated to dryness under nitrogen stream at 40°C and dissolved with 0.5 ml of acetonitrile.

TABLE 2. *Longitude and latitude of sampling sites at 0.25 mi (a sites) and 0.35 mi (b sites)*

Site	Longitude	Latitude
7b	14°17′33.26″	42°25′42.68″
7a	14°17′28.77″	42°25′37.84″
6a	14°21′41.73″	42°23′21.44″
5b	14°25′39.05″	42°20′29.42″
5a	14°25′33.98″	42°20′23.79″
4a	14°29′41.56″	42°16′59.00″
3b	14°33′6.22″	42°14′19.03″
3a	14°33′1.88″	42°14′13.51″
2b	14°37′23.59″	42°12′18.31″
2a	14°37′19.43″	42°12′13.32″
1b	14°42′21.05″	42°10′46.07″
1a	14°42′15.25″	42°10′39.30″
8a	14°43′37.51″	42°07′53.31″
9a	14°44′21.49″	42°05′35.07″

Determination of PAHs: instrument analysis. High-performance liquid chromatography was performed with an HPLC-10ATVP (Shimadzu, Kyoto, Japan) equipped with a multisolvent delivery system, an autosampler (SIL10ADVP), a thermostatic column oven (CTO10ASVP), and a fluorimetric detector (RF10AXLVP). The mobile phase gradient used to elute the PAHs consisted of acetonitrile (solvent A) and water (solvent B) under the following conditions: 80% A for 2 min, 100% A for 25 min, 80% A over 2 min, and then 80% A for 14 min. The flow rate was 0.7 ml/min, the column temperature was 25°C, and the injection volume was 10 μl. The fluorescence detector excitation wavelength was set to 255 nm, and the emission wavelength was 450 nm. The analytical column was a ZORBAX Eclipse PAH Rapid Resolution (4.6 by 100 mm, 1.8 μm; Agilent). The blank samples consisted of clams previously assayed and tested negative for the PAHs under investigation. Blank samples were spiked with 2 and 5 μg/kg PAHs according to the maximum allowable concentrations listed in EC Regulation 1881/2006 *(8)*. The QPH072BT reference material containing all PAHs of interest also was analyzed.

Determination of heavy metals: reagents and standards. Single element 1,000 mg/liter stock standard solutions of Pb, Cd, Hg, rhenium (Re) of trace analysis grade, and 99% $AuCl_3$ (Sigma-Aldrich, St. Gallen, Switzerland) were prepared with high-purity water of 18.2 MΩcm resistivity (PURELAB Option-Q Elga

TABLE 3. *Parameters for microwave oven program*

Step	Power (W)	Ramp (min)	Duration (min)	Venting (min)
1	450	1	4	1
2	800	5	8	1
3	1,000	5	15	1
4	0		15	3

Veolia, Pordenone, Italy). Reagents used for the sample digestion were HNO_3 (≥69%; TraceSELECT, Sigma-Aldrich, Lyon, France) and H_2O_2 (Suprapur 30%; Merck, Darmstadt, Germany).

Matrix modifiers were prepared from 10% $NH_4H_2PO_4$, 1% Pd (PerkinElmer, Shelton, CT), and 1% Mg as $Mg(NO_3)_2$ (Sigma-Aldrich Switzerland) stock solutions by diluting with 0.1% HNO_3. Argon 5.5 of 99.9995% purity was supplied by Sapio (Monza, Italy).

Determination of heavy metals: sample preparation. Before analysis, all the equipment intended to come into direct contact with the sample and all glassware were treated with 1% HNO_3 and rinsed with high-purity water.

About 1.2 g of homogenized sample was weighed into polytetrafluoroethylene vessels and added to 5 ml of 69% HNO_3 and 1 ml of 30% H_2O_2. Mineralization was performed in a Multiwave 3000 microwave digestion system (Anton Paar, Graz, Austria) according to the program shown in Table 3. After cooling, the resulting clear solutions were quantitatively transferred into decontaminated volumetric flasks and diluted to 20 ml with high-purity water. Pb and Cd were analyzed with a graphite furnace atomic absorption spectrometer (GF-AAS; AAnalyst 800, Perkin-Elmer), and Hg was analyzed with an inductively coupled plasma mass spectrometer (ICP-MS; Elan DRCII, PerkinElmer, Concord, Ontario, Canada).

Spiked samples, blank solutions, and the reference material were prepared in the same way.

The analytical quality control was verified using spiked samples and a reference material (fish muscle) supplied by the National Reference Laboratory for Heavy Metals in Food (Istituto Superiore di Sanità, Rome, Italy) containing Pb (0.3700 ± 0.1374 mg/kg), Cd (0.0750 ± 0.0340 mg/kg), and Hg (0.2220 ± 0.0890 mg/kg).

Determination of heavy metals: instrument analysis. For GF-AAS, the test solution was obtained by pressure digestion and atomized in a graphite furnace. The instrumental operating parameters are listed in Table 4. The AAnalyst 800 spectrometer uses Zeeman-effect background correction and a transversely heated graphite atomizer, which provides uniform temperature distribution across the entire length of the graphite tube and has an integrated L'vov platform to overcome potential chemical interference effects. The samples were quantified by the method of standard additions using four-point calibration curves of 10 to 30 μg/liter for Pb and 1 to 3 μg/liter for Cd.

Matrix modifiers were added automatically to each solution (standards, blanks, and samples) by the AS 800 autosampler.

The ICP-MS was used for Hg determination. Sample was introduced with a peristaltic pump connected to a Meinhard nebulizer with a cyclonic spray chamber. The RF power was set to 1,300 W, the plasma gas (Ar) flow was 15 liters/min, and the nebulizer gas (Ar) flow was 0.93 liters/min. The standard addition calibration method was used to quantify the element of interest using the isotope ^{199}Hg. Six standard additions of 1 to 50 μg/liter were made, and Re was used as an internal standard at 10 μg/liter in calibrates and samples to compensate for any random fluctuations of the signals.

Diluted solutions were prepared with the same HNO_3 concentration of calibration standards (1 to 2%, vol/vol). To avoid memory effects for Hg in the sample introduction system (tube of peristaltic pump, nebulizer, and spray chamber) after each solution injected, the system was rinsed with 2 mg/liter $AuCl_3$ for 45 s. The instrument operating parameters are listed in Table 5.

Determination of PCDDs, PCDFs, DL-PCBs, and NDL-PCBs: reagents and standards. All solvents (toluene, n-hexane, dichloromethane, acetone, and isooctane) were organic residue analysis quality (Sigma-Aldrich, Milan, Italy). Prepacked multilayer silica, alumina, and carbon columns were obtained from Fluid Management Systems (Lexington, KY). All standard solutions were supplied by Wellington Laboratories (Guelph, Ontario, Canada).

Calibration solutions DF-CVS CS1 through CS4, $^{13}C_{12}$-labeled internal standard DF-LCS-C200, and recovery standard DF-IS-J were used for PCDD and PCDF analysis. Calibration solutions WP-CVS CS1 through CS7, $^{13}C_{12}$-labeled internal standard WP-LCS, and recovery standard P48-RS-STK were prepared for DL-PCB analysis. Calibration solutions P48-M-CVS CS1 through CS5, $^{13}C_{12}$-labeled internal standard P48-M-ES, and recovery standard P48-RS-STK were selected for NDL-PCB analysis.

Determination of PCDDs, PCDFs, DL-PCBs, and NDL-PCBs: sample preparation. Samples were analyzed by methods (EN 17025) accredited by the International Organization for Standardization (Geneva, Switzerland) and routinely used for monitoring 17 PCDD and PCDF, 12 DL-PCB, and 6 NDL-PCB congeners in food based on the U.S. Environmental Protection Agency (Washington, DC) methods 1613 B and 1668 B. The

TABLE 4. *Instrument parameters for GF-AAS*

			Furnace program temp (°C); ramp, hold (s)			
Element	Parameter	Value	Drying	Ashing	Atomization	Cleaning out
Lead	Wavelength (nm)	283.3	130; 15, 30	850; 10, 20	1,600; 0, 3	2,450; 1, 3
	Slit width (nm)	0.7				
	Signal measurement	Peak height				
	Lamp current (mA)	10				
Cadmium	Wavelength (nm)	228.8	130; 15, 30	350; 10, 20	1,500; 0, 5	2,450; 1, 3
	Slit width (nm)	0.7				
	Signal measurement	Peak height				
	Lamp current (mA)	4				

TABLE 5. *Instrument parameters for ICP-MS*

Parameter	Value
Element	Mercury (^{199}Hg)
Internal standard	Rhenium (^{187}Re)
Measurement	Standard mode
Spray chamber	Cyclonic
Nebulizer	Meinhard
Sample uptake (ml/min)	1
RF power (W)	1,300
Plasma Ar flow (liters/min)	15
Lens voltage	7.50
Nebulizer Ar flow (liters/min)	0.93
Auxiliary Ar flow (liters/min)	1.20
Mathieu parameters	
RPq	0.25
RPa	0
Dwell time (ms)	200
Sweeps per reading	25
Replicates	4
Delay time (s)	30
Wash time (s)	45

analytical methods have been successfully verified in many proficiency tests over 10 years.

Before the analysis, each sample (~15 g) was mixed with a quantity of diatomaceous earth two to three times greater than the weight of the sample aliquot and kept overnight in an oven at 40°C.

A mixture of internal standards ($^{13}C_{12}$-labeled congeners of 17 PCDD and PCDFs, 12 DL-PCBs, and 6 NDL-PCBs) was added to each sample. The samples were processed by accelerated solvent extraction with *n*-hexane–acetone (80:20, vol/vol) using a Dionex ASE 350 (Thermo Fisher Scientific, Waltham, MA) at 1,500 psi and 125°C because this pressure increased the boiling points of hexane and acetone.

After solvent evaporation under vacuum at 40°C, the extract was dissolved in hexane and subjected to liquid-liquid partitioning with concentrated sulfuric acid, 20% aqueous potassium hydroxide, and saturated aqueous sodium chloride. The extract was then purified with an automated clean-up process (Power-Prep, Fluid Management Systems) using disposable columns (multilayer silica, alumina, and carbon). The fraction containing the DL-PCBs and NDL-PCBs was collected after elution from the alumina column, and the fraction containing the PCDDs and PCDFs was eluted from the carbon column.

The two fractions were concentrated, first under vacuum and then under nitrogen stream, and the remainders were dissolved in the corresponding recovery standards solutions ($^{13}C_{12}$-labelled congeners of PCDDs and PCDFs and PCBs, different from those added at the beginning of the process).

A laboratory blank and a control sample were analyzed for each batch of 5 and 10 samples, respectively.

Determination of PCDDs, PCDFs, DL-PCBs, and NDL-PCBs: instrument analysis. The measurements were performed on two Trace Series 2000 gas chromatographs coupled to a MAT 95 XP and a MAT 95 XL (Thermo Fisher, Bremen, Germany).

PCDDs and PCDFs were analyzed by gas chromatography (GC) on a DB-5 MS capillary column (60 m by 0.25 mm, 0.10-μm film thickness; J&W Scientific, Folsom, CA) and determined by high-resolution mass spectrometry (HRMS) at a resolution of 10,000 operating with electron ionization at 40 eV in the selected ion monitoring mode. DL-PCBs and NDL-PCBs were separated by HRGC on a HT8-PCB capillary column (60 m by 0.25 mm, 0.25-μm film thickness; SGE Analytical Science, Ringwood, Victoria, Australia) and determined by HRMS using the same operating conditions adopted for PCDDs and PCDFs.

TABLE 6. *Concentrations of lead and cadmium in samples of* Chamelea gallina *collected 0.25 and 0.35 mi off the coast*

	Lead (mg/kg wet wt)		Cadmium (mg/kg wet wt)	
Site	0.25 mi	0.35 mi	0.25 mi	0.35 mi
1	0.140	0.120	0.140	0.140
2	0.062	0.140	0.077	0.120
3	0.059	0.036	0.110	0.120
4	0.110		0.066	
5	<0.027	0.110	0.110	0.088
6	0.160		0.074	
7	0.110	0.090	0.070	0.075
8	0.120		0.098	
9	0.180		0.250	

Toxic equivalent quotient (WHO-TEQ) values for PCDDs, PCDFs, and DL-PCBs were calculated using the toxic equivalent factor model proposed by the World Health Organization in 2005 *(36)*. For NDL-PCBs, the concentrations were expressed in nanograms per gram of wet weight.

WHO-TEQs and the sum of six NDL-PCBs were calculated as upper bound concentrations, assuming that values for specific PCDD, PCDF, and PCB congeners that were below the limit of quantification were equal to the respective limits. The analytical uncertainty was ±20% for WHO-TEQs and sum of the six NDL-PCBs.

Statistical analysis. Principal component analysis (PCA) was performed using the statistical software STATISTICA for Windows (STAT version 8.0, StatSoft, Tulsa, OK).

RESULTS AND DISCUSSION

In the present study, 250 *C. gallina* individuals were collected from the Abruzzi region coast and analyzed for contaminants with regulatory limits: PAHs, heavy metals (lead, cadmium, and total mercury), NDL-PCBs, PCDDs, PCDFs, and DL-PCBs.

For PAHs and total mercury, all samples had concentrations below the detection limits (benzo[*a*]anthracene < 0.18 μg/kg, chrysene < 0.30 μg/kg, benzo[*b*]fluoranthene < 0.12 μg/kg, benzo[*a*]pyrene < 0,08 μg/kg, and Hg < 0.0050 mg/kg). The concentrations of the other investigated metals are listed in Table 6. Lead and cadmium concentrations in samples collected at 0.25 mi ranged from the quantification limit to 0.180 mg/kg wet weight and from 0.070 to 0.250 mg/kg wet weight, respectively. The mean concentrations were 0.104 mg/kg wet weight for lead and 0.110 mg/kg wet weight for cadmium. The samples collected at 0.35 mi had concentrations similar to those for samples collected at 0.25 mi, with means of 0.099 mg/kg wet weight and 0.108 mg/kg wet weight for lead and cadmium, respectively. The highest concentrations were detected in samples from sites 9a and 1a, corresponding to the sampling area in the southern part of the Abruzzi region. However, the regulatory limits of 1.5 mg/kg wet weight for lead and 1.0 mg/kg wet weight for cadmium were never exceeded.

TABLE 7. *Concentrations of NDL-PCBs in samples of* Chamelea gallina *collected 0.25 mi off the coast*

	Concn (ng/g wet wt) at sites:								
Congener	1a	2a	3a	4a	5a	6a	7a	8a	9a
PCB-28	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
PCB-52	0.08	0.09	0.08	0.08	0.08	0.08	0.08	0.09	0.09
PCB-101	0.20	0.22	0.20	0.21	0.19	0.22	0.22	0.22	0.23
PCB-138	0.42	0.47	0.44	0.41	0.40	0.48	0.43	0.49	0.46
PCB-153	0.72	0.86	0.85	0.79	0.74	0.84	0.81	0.89	0.87
PCB-180	0.44	0.48	0.46	0.43	0.42	0.47	0.46	0.49	0.48
Total	1.88	2.14	2.05	1.94	1.85	2.11	2.02	2.20	2.15

TABLE 8. *Concentrations of DL-PCBs in samples of* Chamelea gallina *collected 0.25 mi off the coast*

		Concn (pg/g wet wt) at sites:								
Congener	TEF[a]	1a	2a	3a	4a	5a	6a	7a	8a	9a
PCB-77	0.0001	23.44	71.77	32.45	21.17	20.08	30.09	22.28	20.70	33.52
PCB-81	0.0003	<1.36	<28.22	<1.06	<1.26	<1.30	<1.39	<1.63	<1.44	<1.76
PCB-126	0.1	1.03	0.85	0.69	1.03	0.92	0.97	1.01	1.02	1.02
PCB-169	0.03	<0.05	<0.09	<0.06	<0.09	<0.15	<0.10	<0.08	<0.17	<0.12
PCB-105	0.00003	40.46	42.63	42.05	39.66	37.16	43.0	40.37	43.80	44.13
PCB-114	0.00003	3.52	4.82	3.60	3.31	3.25	3.24	3.25	3.57	3.84
PCB-118	0.00003	118.42	123.48	124.65	117.67	110.84	127.77	123.71	130.86	131.36
PCB-123	0.00003	2.70	2.65	2.54	2.62	2.43	2.50	2.59	2.51	2.80
PCB-156	0.00003	31.55	34.12	33.25	31.39	30.12	34.19	32.63	34.51	35.27
PCB-157	0.00003	7.57	7.61	7.31	7.17	6.78	7.81	7.39	8.01	7.85
PCB-167	0.00003	18.63	19.73	19.31	18.05	17.27	19.37	19.08	19.92	20.24
PCB-189	0.00003	5.06	5.97	5.91	5.34	5.36	5.74	5.70	6.05	6.20
Sum of WHO-TEQs for DL-PCBs		0.114	0.111	0.082	0.115	0.106	0.111	0.114	0.117	0.117

[a] TEF, toxic equivalency factor.

TABLE 9. *Concentrations of PCDDs and PCDFs in samples of* Chamelea gallina *collected 0.25 mi off the coast*

		Concn (pg/g wet wt) at sites:								
Congener	TEF[a]	1a	2a	3a	4a	5a	6a	7a	8a	9a
2,3,7,8-T4CDD	1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.006	<0.001	<0.001
1,2,3,7,8-P5CDD	1	<0.015	<0.001	0.017	<0.007	0.026	<0.001	<0.001	0.011	<0.001
1,2,3,4,7,8-H6CDD	0.1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
1,2,3,6,7,8-H6CDD	0.1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
1,2,3,7,8,9-H6CDD	0.1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
1,2,3,4,6,7,8-H7CDD	0.01	0.030	<0.003	0.055	0.017	0.014	<0.003	<0.001	0.035	0.010
O8CDD	0.0003	0.171	0.195	0.153	0.198	0.160	0.220	0.089	0.172	0.206
2,3,7,8-T4CDF	0.1	0.170	0.153	0.135	0.181	0.159	0.126	0.161	0.143	0.153
1,2,3,7,8-P5CDF	0.03	0.011	0.017	0.010	0.020	0.012	0.010	0.009	0.011	0.012
2,3,4,7,8-P5CDF	0.3	0.009	0.017	0.022	0.036	0.023	0.032	0.022	0.029	0.061
1,2,3,4,7,8-H6CDF	0.1	<0.006	<0.002	0.007	<0.001	<0.001	0.011	<0.004	<0.001	<0.001
1,2,3,6,7,8-H6CDF	0.1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.013	<0.002	<0.001
1,2,3,7,8,9-H6CDF	0.1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.002	<0.001	<0.001	<0.001
2,3,4,6,7,8-H6CDF	0.1	<0.001	<0.001	0.007	<0.001	<0.001	<0.001	<0.001	<0.001	<0.003
1,2,3,4,6,7,8-H7CDF	0.01	0.014	0.022	0.014	0.012	<0.001	0.019	<0.005	<0.001	<0.001
1,2,3,4,7,8,9-H7-CDF	0.01	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
O8CDF	0.0003	0.034	0.023	0.014	0.031	0.028	0.031	<0.001	0.017	<0.010
Sum of WHO-TEQs for PCDDs and PCDFs		0.037	0.024	0.041	0.038	0.051	0.027	0.032	0.036	0.037

[a] TEF, toxic equivalency factor.

TABLE 10. *Concentrations of NDL-PCBs in samples of* Chamelea gallina *collected 0.35 mi off the coast*

Congener	Concn (ng/g wet wt) at sites:				
	1b	2b	3b	5b	7b
PCB-28	0.02	0.02	0.02	0.02	0.02
PCB-52	0.09	0.08	0.07	0.08	0.08
PCB-101	0.25	0.22	0.19	0.21	0.22
PCB-138	0.54	0.46	0.43	0.48	0.44
PCB-153	0.98	0.85	0.80	0.85	0.88
PCB-180	0.55	0.49	0.45	0.52	0.48
Total	2.43	2.12	1.96	2.16	2.12

Cardelicchio et al. *(3)* found higher concentrations of cadmium, lead, and mercury in samples of *Mytilus galloprovincialis* collected along the Apulian coast. Higher concentrations of lead were also found in mussels from the northern Adriatic Sea (Venice Lagoon) and the Croatian coasts *(15, 19, 30)*. Kljaković-Gašpić et al. *(19)* found higher cadmium levels in mussels collected from the Croatian coasts. Although the *C. gallina* samples evaluated in the present study were not dangerous for a moderate mollusk consumer based on their concentrations of heavy metals, depuration of the clams could be used as a preventive action. In a recent study, lead and cadmium concentrations decreased by 23 and 75%, respectively, after 24 h of depuration *(7)*.

TABLE 11. *Concentrations of DL-PCBs in samples of* Chamelea gallina *collected 0.35 mi off the coast*

Congener	TEF[a]	Concn (pg/g wet wt) at sites:				
		1b	2b	3b	5b	7b
PCB-77	0.0001	34.53	33.27	28.04	26.29	32.41
PCB-81	0.0003	<1.47	<1.60	<1.02	<1.37	<1.35
PCB-126	0.1	0.89	0.85	0.92	0.98	0.98
PCB-169	0.03	<0.15	<0.24	<0.13	<0.23	<0.16
PCB-105	0.00003	50.74	44.35	41.11	45.88	43.07
PCB-114	0.00003	4.19	3.80	3.38	3.88	3.63
PCB-118	0.00003	148.41	131.19	120.56	136.75	129.86
PCB-123	0.00003	2.90	2.60	2.30	2.64	2.49
PCB-156	0.00003	39.98	35.78	33.68	36.80	35.23
PCB-157	0.00003	9.21	8.22	7.53	8.30	7.89
PCB-167	0.00003	23.55	21.08	19.13	21.28	20.59
PCB-189	0.00003	7.30	6.06	5.64	6.15	6.04
Sum of WHO-TEQ for DL-PCBs		0.105	0.103	0.106	0.116	0.114

[a] TEF, toxic equivalency factor.

TABLE 12. *Concentrations of PCDDs and PCDFs in samples of* Chamelea gallina *collected 0.35 mi off the coast*

Congener	TEF[a]	Concn (pg/g wet wt) at sites:				
		1b	2b	3b	5b	7b
2,3,7,8-T4CDD	1	<0.001	<0.001	<0.001	<0.001	<0.001
1,2,3,7,8-P5CDD	1	<0.011	<0.001	<0.001	<0.003	0.009
1,2,3,4,7,8-H6CDD	0.1	<0.001	<0.001	<0.001	<0.001	<0.001
1,2,3,6,7,8-H6CDD	0.1	0.014	<0.002	<0.001	<0.001	<0.001
1,2,3,7,8,9-H6CDD	0.1	<0.001	<0.002	<0.001	<0.001	<0.002
1,2,3,4,6,7,8-H7CDD	0.01	0.082	0.024	0.029	0.017	0.052
O8CDD	0.0003	0.175	0.250	0.130	0.233	0.143
2,3,7,8-T4CDF	0.1	0.202	0.216	0.167	0.171	0.164
1,2,3,7,8-P5CDF	0.03	0.012	0.013	0.007	0.015	0.025
2,3,4,7,8-P5CDF	0.3	0.041	0.011	0.017	0.042	0.041
1,2,3,4,7,8-H6CDF	0.1	<0.001	<0.001	0.008	<0.001	<0.001
1,2,3,6,7,8-H6CDF	0.1	<0.001	<0.001	0.005	<0.001	<0.001
1,2,3,7,8,9-H6CDF	0.1	<0.002	<0.001	<0.001	<0.001	<0.001
2,3,4,6,7,8-H6CDF	0.1	<0.001	<0.001	0.007	0.007	0.011
1,2,3,4,6,7,8-H7CDF	0.01	0.019	<0.002	0.029	0.013	0.015
1,2,3,4,7,8,9-H7-CDF	0.01	<0.006	<0.002	<0.001	<0.001	<0.001
O8CDF	0.0003	0.017	<0.001	0.029	0.021	0.030
Sum of WHO-TEQs for PCDDs and PCDFs		0.049	0.029	0.027	0.036	0.042

[a] TEF, toxic equivalency factor.

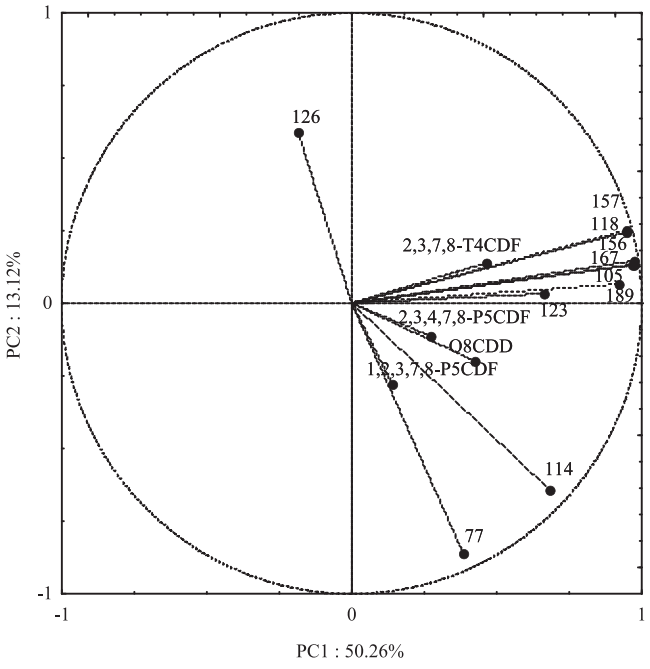


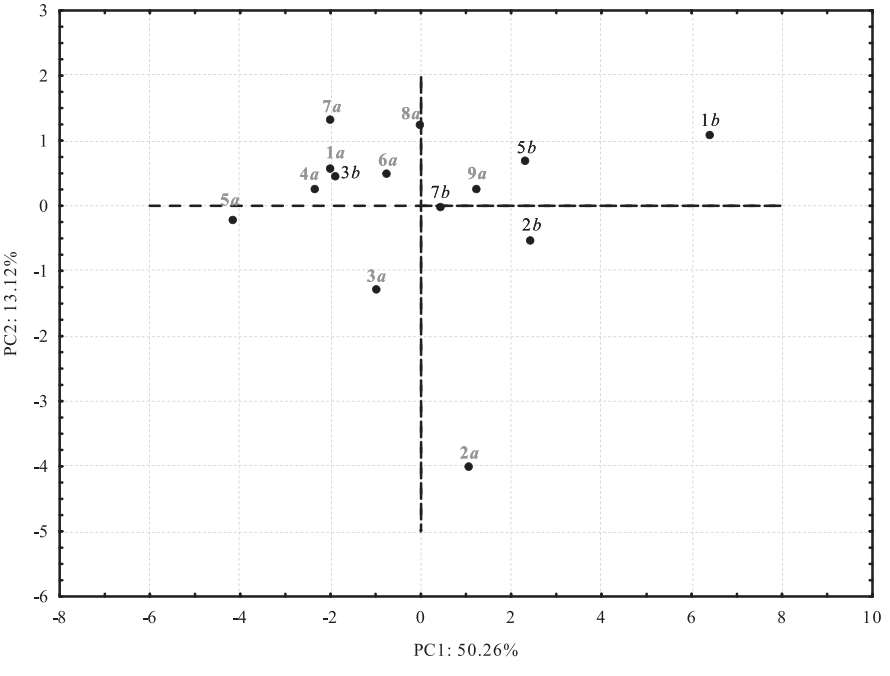
FIGURE 2. *Principal component analysis (PCA) scores for distribution of dioxin-like PCB and dioxin congeners.*

The NDL-PCB, DL-PCB, PCDD, and PCDF concentrations in samples collected at 0.25 mi are listed in Tables 7 through 9, and the data obtained for samples collected at 0.35 mi are listed in Tables 10 through 12. No sample had concentrations that exceeded the regulatory limits. The NDL-PCBs profile was in all cases dominated by PCB-153, followed by PCB-180 and PCB-138. This predominance was in accordance with findings by other authors *(4)* because the highly chlorinated congeners are not efficiently metabolized by mollusks and easily accumulate in their lipids *(6)*. The concentrations of PCB-28 and PCB-52 were comparable in all the sampling areas. Regarding DL-PCBs, the congener PCB-118 was the most representative. Other authors reported that PCB-118 was the predominant congener in mussels collected in the Istanbul Strait *(29)*.

Figures 2 and 3 show the PCA results for the variation and distribution of PCB congeners at the sampling sites at 0.25 mi (1a to 9a) and 0.35 mi (1b, 2b, 3b, 5b, and 7b). PC1 and PC2 captured 63.38% of the total data variability. The PC values were similar for the majority of sampling sites except 2a and 1b. PCB-77 (71.77 pg/g wet weight) and PCB-114 (4.82 pg/g wet weight) were the most abundant congeners at site 2a, whereas all other DL-PCB congeners were more expressed at site 1b. For the PCDD and PCDF congeners, no significant differences were observed among the sampling sites; O8CDD and 2,3,7,8-T4CDF were the predominant congeners, and 2,3,7,8-T4CDF was the highest contributor to the TEQ.

The variability in PCB concentrations among the sampling sites was likely due to whether the specific location was close to industrial or urbanized areas *(23)*. Because the Adriatic Sea is semienclosed, the pollution in marine areas is mainly found where sedimentary materials are deposited from rivers, resulting in an accumulation of easily exchangeable pollutants. The central Adriatic Sea is characterized by intermittent fluxes of dense waters, whereas in the eastern sea high-salinity water coming from the south moves in the opposite direction. However, the central Adriatic Sea is less affected by pollution effluents than is the northern sea, where the Po River drains a very industrialized and intensively cultivated area *(14)*. In the present study, DL-PCB, PCDD, and PCDF concentrations were lower than those reported by other authors in mollusks (mussel and clam) from other coasts along the northern, central, and southern Adriatic Sea *(2, 26)*. Results from a recent assessment of environmental quality status of the three Italian subregions (Adriatic Sea, west Mediterranean Sea, and Ionian and central Mediterranean Sea) selected for the Marine Strategy Framework Directive confirmed that the

FIGURE 3. *Principal component analysis (PCA) loads among sampling sites (a, samples collected at 0.25 mi; b, samples collected at 0.35 mi).*



mussels coming from the Adriatic Sea subregion have never exceeded the maximum limits set by Regulation EC No 1881/2006 and its updates *(22)*.

Seafood is probably the main source of human exposure to some pollutants, such as heavy metals, dioxins, and dioxin-like compounds, and coastal waters are usually the receptors of particles carried by rivers to the marine environment. This study was conducted to evaluate the concentrations of contaminants bioaccumulated by a bivalve mollusk that is commonly consumed in Mediterranean countries and to evaluate the potential toxicity of this clam for consumers. The results revealed that *C. gallina* from the central Adriatic Sea could be considered safe with regard to the contaminants studied; however, the concentrations of these contaminants should be monitored periodically with respect to consumer health.

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REFERENCES

1. Angioni, S. A., C. Giansante, and N. Ferri. 2010. The clam (*Chamelea gallina*): evaluation of the effects of solids suspended in seawater on bivalve mollusks. *Vet. Ital.* 46:101–106.
2. Bayarri, S., L. Turrio Baldassarri, N. Iacovella, F. Ferrara, and A. di Domenico. 2001. PCDDs, PCDFs, PCBs and DDE in edible marine species from the Adriatic Sea. *Chemosphere* 43:601–610.
3. Cardelicchio, N., A. Buccolieri, A. Di Leo, S. Giandomenico, and L. Spada. 2008. Levels of metals in reared mussels from Taranto Gulf (Ionian Sea, southern Italy). *Food Chem.* 107:890–896.
4. Carro, N., I. García, M. Ignacio, and A. Mouteira. 2010. Spatial and temporal trends of PCBs (polychlorinated biphenyls) in mussel from Galician coast (1998–2008). *Environ. Int.* 36:873–879.
5. Cordier, S., A. Lehébel, E. Amar, L. Anzivino-Viricel, M. Hours, C. Monfort, C. Chevrier, M. Chiron, and E. Robert-Gnansia. 2010. Maternal residence near municipal waste incinerators and the risk of urinary tract birth defects. *Occup. Environ. Med.* 67:493–499.
6. Dodoo, D. K., D. K. Essumang, and J. W. A. Jonathan. 2013. Accumulation profile and seasonal variations of polychlorinated biphenyls (PCBs) in bivalves *Crassostrea tulipa* (oysters) and *Anadara senilis* (mussels) at three different aquatic habitats in two seasons in Ghana. *Ecotoxicol. Environ. Saf.* 88:26–34.
7. El-Gamal, M. M. 2011. The effect of depuration on heavy metals, petroleum hydrocarbons, and microbial contamination levels in *Paphia undulata* (Bivalvia: Veneridae). *Czech J. Anim. Sci.* 56:345–354.
8. European Commission. 2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum level for certain contaminants in foodstuffs. *Off. J. Eur. Union* L 364:5–24.
9. European Commission. 2008. Directive (EC) No 56/2008 of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). *Off. J. Eur. Union* L 164:19–40.
10. European Commission. 2011. Commission Regulation (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. *Off. J. Eur. Union* L 215:4–8.
11. European Commission. 2011. Commission Regulation (EU) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non dioxin-like PCBs in foodstuffs. *Off. J. Eur. Union* L 320:18–23.
12. European Food Safety Authority. 2005. Scientific opinion of the panel on contaminants in the food chain on a request from the Commission related to the presence of non dioxin-like polychlorinated biphenyls (PCB) in feed and food. *EFSA J.* 284:1–137.
13. European Food Safety Authority. 2009. Scientific opinion of the panel on contaminants in the food chain on a request from the European Commission on cadmium in food. *EFSA J.* 980:1–139.
14. Frascari, F., M. Frignani, S. Guerzoni, and M. Ravaioli. 1988. Sediments and pollution in the northern Adriatic Sea. *Ann. N. Y. Acad. Sci.* 534:1000–1020. doi:10.1111/j.1749-6632.1988.tb30191.x.
15. Giusti, L., and H. Zhang. 2002. Heavy metals and arsenic in sediments, mussels and marine water from Murano (Venice, Italy). *Environ. Geochem. Health* 24:47–65.
16. Imura, H., T. Yamada, K. Mishima, K. Fujiwara, H. Kawaki, A. Hirata, N. Sogawa, T. Ueno, and T. Sugahara. 2010. Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin suggests abnormal palate development after palatal fusion. *Congenit. Anom. (Kyoto)* 50:77–84.
17. International Agency for Research on Cancer. 1997. Polychlorinated dibenzo-para-dioxins and polychlorinated dibenzofurans. IARC monographs on the evaluation of carcinogenic risks to humans, vol. 69. International Agency for Research on Cancer, Lyon, France.
18. International Agency for Research on Cancer. 2012. Chemical agents and related occupations: a review of human carcinogens. IARC monographs on the evaluation of carcinogenic risks to humans, vol. 100F. International Agency for Research on Cancer, Lyon, France.
19. Kljaković-Gašpić, Z., S. Herceg-Romanić, D. Kožul, and J. Veža. 2010. Biomonitoring of organochlorine compounds and trace metals along the eastern Adriatic coast (Croatia) using *Mytilus galloprovincialis*. *Mar. Pollut. Bull.* 60:1879–1889.
20. Kurt, P. B., and H. B. Ozkoc. 2004. A survey to determine levels of chlorinated pesticides and PCBs in mussels and seawater from the mid–Black Sea coast of Turkey. *Mar. Pollut. Bull.* 48:1076–1083.
21. Lundqvist, C., M. Zuurbier, M. Leijs, C. Johansson, S. Ceccatelli, M. Saunders, G. Schoeters, G. Ten Tusscher, and J. G. Koppe. 2006. The effects of PCBs and dioxins on child health. *Acta Paediatr.* 95(453):S55–S64.
22. Maggi, C., S. Lomiri, B. Di Lorenzo, M. d'Antona, and M. T. Berducci. 2014. Environmental quality of Italian marine water by means of marine strategy framework directive (MSFD) descriptor 9. *PloS One* 9(9):e108463.
23. Martini, P., G. Castellari, and C. R. Ferrari. 2008. Comparative analysis of bioaccumulation of pollutants in some levels of marine trophic web. *Biol. Mar. Mediterr.* 15:118–121.
24. Matozzo, V., M. Monari, J. Foschi, O. Cattani, G. P. Serrazanetti, and M. G. Marin. 2009. First evidence of altered immune responses and resistance to air exposure in the clam *Chamelea gallina* exposed to benzo(a)pyrene. *Arch. Environ. Contam. Toxicol.* 56:479–488.
25. Matozzo, V., M. Monari, J. Foschi, G. P. Serrazanetti, O. Cattani, and M. G. Marin. 2007. Effects of salinity on the clam *Chamelea gallina*. Part I. Alterations in immune responses. *Mar. Biol.* 151:1051–1058.
26. Micheletti, C., A. Critto, and A. Marcomini. 2007. Assessment of ecological risk from bioaccumulation of PCDD/Fs and dioxin-like PCBs in a coastal lagoon. *Environ. Int.* 33:45–55.
27. Monari, M., J. Foschi, R. Rosmini, M. G. Marin, and G. P. Serrazanetti. 2011. Heat shock protein 70 response to physical and chemical stress in *Chamelea gallina*. *J. Exp. Mar. Biol. Ecol.* 397:71–78.
28. Moschino, V., and M. G. Marin. 2006. Seasonal changes in physiological responses and evaluation of "well-being" in the Venus clam *Chamelea gallina* from the northern Adriatic Sea. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 145:433–440.
29. Okay, O. S., B. Karacık, S. Başak, B. Henkelmann, S. Bernhöft, and K. W. Schramm. 2009. PCB and PCDD/F in sediments and mussels of the Istanbul Strait (Turkey). *Chemosphere* 76:159–166.
30. Orescanin, V., I. Lovrencic, L. Mikelic, D. Barisic, Z. Matasin, S. Lulic, and D. Pezelj. 2006. Biomonitoring of heavy metals and arsenic on the east coast of the Middle Adriatic Sea using *Mytilus*

galloprovincialis. *Nucl. Instrum. Methods Phys. Res. B Beam Interact. Mater. Atoms* 245:495–500.

31. Perugini, M., P. Visciano, M. Manera, M. C. Abete, S. Gavinelli, and M. Amorena. 2013. Contamination of different portions of raw and boiled specimens of Norway lobster by mercury and selenium. *Environ. Sci. Pollut. Res.* 20:8255–8262.
32. Perugini, M., P. Visciano, M. Manera, M. C. Abete, R. Tarasco, and M. Amorena. 2014. Lead, cadmium and chromium in raw and boiled portions of Norway lobster. *Food Addit. Contam. B* 21:1–6.
33. Puga, A. 2011. Perspectives on the potential involvement of the AH receptor-dioxin axis in cardiovascular disease. *Toxicol. Sci.* 120:256–261.
34. Schecter, A., L. Birnbaum, J. J. Ryan, and J. D. Constable. 2006. Dioxins: an overview. *Environ. Res.* 101:419–428.
35. Van den Berg, M., L. Birnbaum, A. T. C. Bosveld, B. Brunstrom, P. Cook, M. Feely, J. P. Giesy, A. Hanberg, R. Hasegawa, S. W. Kennedy, T. Kubiak, J. C. Larsen, F. X. R. van Leeuwen, A. K. D. Liem, C. Nolt, R. E. Peterson, L. Poellinger, S. Safe, D. Schrenk, D. Tillit, M. Tysklind, M. Younes, F. Waern, and T. Zacharewski. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ. Health Perspect.* 106:775–792.
36. Van den Berg, M., L. Birnbaum, M. Denison, M. De Vito, W. Farland, M. Feeley, H. Fiedler, H. Hakansson, A. Hanberg, L. Haws, M. Rose, S. Safe, D. Schrenk, C. Tohyama, A. Tritscher, J. Tuomisto, M. Tysklind, N. Walker, and R. E. Peterson. 2006. The 2005 World Health Organization re-evaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicol. Sci.* 93:223–241.
37. Visciano, P., M. Perugini, M. Manera, and M. Amorena. 2009. Selected polycyclic aromatic hydrocarbons in smoked tuna, swordfish and Atlantic salmon fillets. *Int. J. Food Sci. Technol.* 44:2028–2032.
38. Visciano, P., M. Perugini, M. Manera, C. Salese, G. Martino, and M. Amorena. 2014. Nutritional quality and safety related to trace element content in fish from Tyrrhenian Sea. *Environ. Contam. Toxicol. B* 92:557–561.