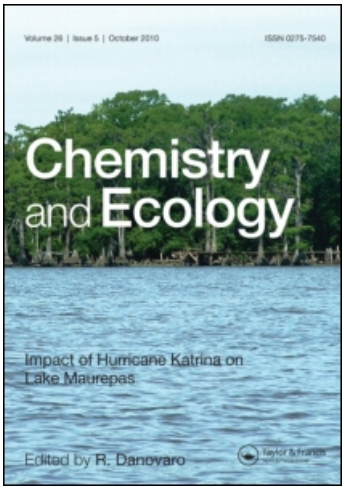

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

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Online publication date: 20 May 2010

To cite this Article Renzi, Monia , Perra, Guido , Lobianco, Arianna , Mari, Elena , Guerranti, Cristiana , Specchiulli, Antonietta , Pepi, Milva and Focardi, Silvano(2010) 'Environmental quality assessment of the marine reserves of the Tuscan Archipelago, Central Tyrrhenian Sea (Italy)', Chemistry and Ecology, 26: 1, 299 — 317
To link to this Article: DOI: 10.1080/02757541003627647
URL: http://dx.doi.org/10.1080/02757541003627647

Chemistry and Ecology
Vol. 26, Supplement, June 2010, 299–317

Taylor & Francis
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Monia Renzi[a,b]*, Guido Perra[b], Arianna Lobianco[b], Elena Mari[a], Cristiana Guerranti[b], Antonietta Specchiulli[c], Milva Pepi[a,b] and Silvano Focardi[a,b]

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(*Received 17 May 2009; final version received 14 December 2009*)

There has been a worldwide increase in the number of Marine Protected Areas and marine reserves over the last decade. In these areas, the protection measures adopted are related to specific management goals; nevertheless, actual knowledge of the effectiveness of the restrictions is far from exhaustive. This article aims to contribute to knowledge of the environmental quality of the marine reserves in the Tuscan Archipelago (Mediterranean central area) which is composed of seven islands at different levels of protection. A monitoring programme spanning multiple years was performed on water and sediment samples to finalise a definition of the trophic levels and the response of microbiological indicators (total heterotrophic bacteria, *Actynomyces*, hydrocarbon-degrading bacteria) to persistent organic pollutants (polychlorinated biphenyls, 1,1-*bis*-(4-chlorophenyl)-2,2-dichloroethene, hexachlorobenzene and polybrominated diphenyl ethers) was assessed. The results showed that these reserves were generally of good quality. A clear fingerprint produced by human activities along the coast and significant differences relating to the level of protection were observed. There exists the need to also consider basin dynamics when planning the protection management strategies adopted for marine reserves.

Keywords: central Tyrrhenian Sea; Mediterranean Sea; marine reserves; sediment; water; persistent organic pollutants; microbiological indicators

1. Introduction

The influence of human activities on marine biota has led to increased conservation efforts in marine systems over the last few decades [1]. As a consequence, the number of Marine Protected Areas and marine reserves has increased worldwide [2] and in the Mediterranean Sea, with an annual growth of 5.2%, the percentage of Marine Protected Areas under national jurisdiction is 1.5 [3]. Marine reserves are a relatively new concept within marine resource management [4]. They represent an area of sea in which legal restrictions are applied to several human activities [5], fulfilling the requirements of modern conservation, and which may be used in synergy with traditional forms of marine resource management [6]. It is generally recognised that reserves can

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ISSN 0275-7540 print/ISSN 1029-0370 online

DOI: 10.1080/02757541003627647
http://www.informaworld.com

provide unique protection for critical areas and a spatial escape for overexploited species [7], also acting as buffer structures against incorrect management [8]. Nevertheless, the effectiveness of the adopted protection measures needs further study [3,9] because they are related to specific management goals. In fact, marine reservoirs shield populations within their confines, but do not guarantee protection against major threats to marine environmental quality: for example, coastal modifications and subsequent changes in local hydrodynamic and sedimentary regimes, disease, the spread of exotic species and direct or indirect chemical pollution [10]. Furthermore, in Europe, the monitoring associated with marine reserve control programmes has been mainly to check the parameters that define water quality for human safety, and has excluded analyses of specific indicators of human activity [11]. However, many studies have shown that Marine Protected Areas, which are subject to the highest level of protection (integral reserves), may be affected by man-related contaminants, such as trace elements and persistent organic pollutants (POPs) [12,13]. The toxicological effects of these pollutants on marine species have been well documented, particularly in adult [14], juvenile or early life stages, gametes and embryos [15], for example, the bioconcentration, bioaccumulation and biomagnification [16–18] properties of algae [19], invertebrates [20] and vertebrates [21]. Marine reserves are clearly characterised by very few local sources of chemicals, but long-range run-off depositions [22], human activities along the coast [23], tourism and fishing [24] represent elements that have to be considered for their ecotoxicological implications and during assessment of the local dynamics between coastal and marine systems. The central Tyrrhenian Sea is an area of the Mediterranean for which there is a lack of bibliographic data related to marine quality assessment and general POP levels, although particular attention has been given to highly polluted sites (harbours [25] and highly industrialised coasts [23]), eutrophicated water (northern Adriatic Sea [26], coastal lagoons [27]) and reserves of international interest (cetaceous sanctuary). The marine reserves of the Tuscan Archipelago (Italy) represent an interesting study area for the central Tyrrhenian Sea because of their geographical location, geomorphologic structure and the different levels of protection adopted for each of the islands composing the Archipelago. The aim of this study is to contribute to the knowledge of water quality in the Archipelago and provide a baseline from which to draw a preliminary picture of these marine reserves. In particular, trophic levels were defined and the response of nonclassical microbiological indicators (total heterotrophic bacteria, *Actynomyces*, hydrocarbon-degrading bacteria) to pollution of human origin in superficial waters was observed. POPs (polychlorinated biphenyls (PCBs), 1,1-*bis*-(4-chlorophenyl)-2,2-dichloroethene (p,p'-DDE), hexachloro benzene (HCB), polycyclic aromatic hydrocarbons (PAHs) and polybrominated diphenyl ether (PBDE)) levels in sediments were also explored to assess the occurrence of recent trends highlighting the impact of coastal and/or local activities, and to evaluate significant differences among the islands that are related to the different protection measures adopted.

2. Materials and methods

2.1. *Study area*

The marine reserves of the Tuscan Archipelago (Figure 1) are located between the island of Corsica (France) and the Tuscan coast, near the urban centres of Orbetello and Livorno. Gorgona is the northernmost island, sited at the border between the central Tyrrhenian Sea and the Ligurian Sea, whereas Giannutri is the southernmost island, close to the Mount Argentario peninsula. The islands are characterised by different levels of protection: zone 1 (integral reserve: no-take, no-access zone) and zone 2 (simple protection). The main sampled characteristics of the islands and their geographical locations are summarised in Table 1, and local sources of pollutants and general water currents are shown in Figure 1.

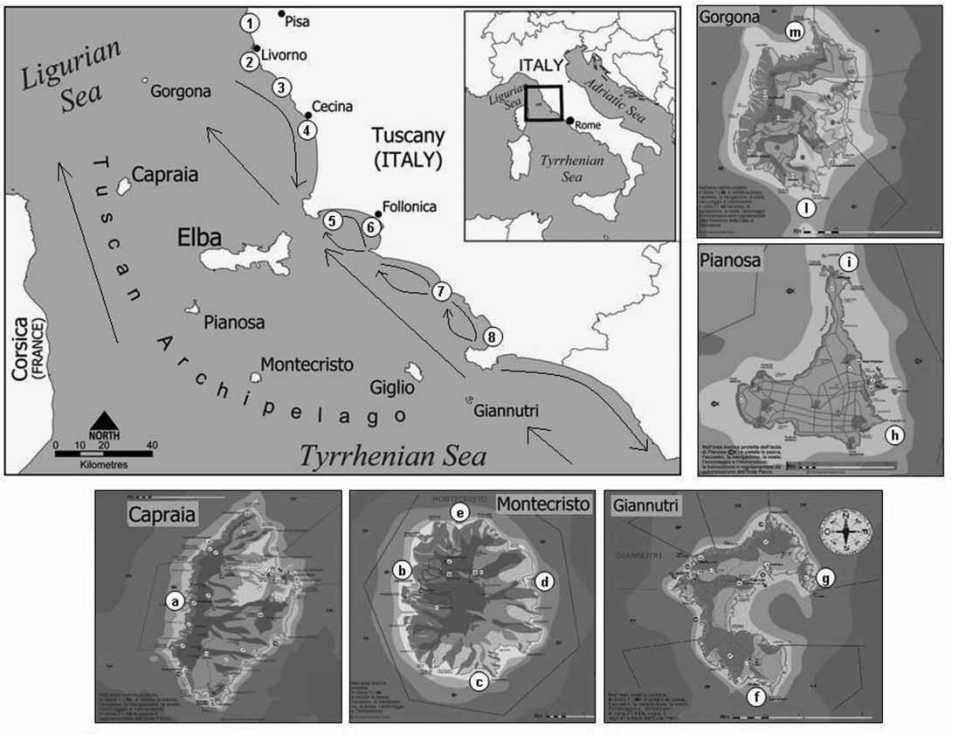

Figure 1. (1) Mouth of the Arno River; (2) site of coastal industry and harbour (Livorno); (3) site of coastal industry (Rosignano-Solvay); (4) mouth of the Cecina River; (5) site of coastal industry (Piombino); (6) site of coastal industry (Follonica); (7) mouth of the Ombrone River; (8) mouth of the Albegna River. Sampling sites – Isola di Capraia: (a) Punta del Fondo; Isola di Montecristo: (b) Cala Maestra, (c) Cala Grande, (d) Cala dello Scoglio, (e) Cala del Santo; Isola di Giannutri: (f) Grottoni, (g) Cala dello Scoglio; Isola di Pianosa: (h) Punta Secca, (i) Isolotto 'la Scarpa'; Isola di Gorgona: (l) Punta di Cala Scirocco, (m) Punta di Cala Maestra. Arrows show general water currents.

Table 1. Main charateristics of the sampled islands.

Island	Sampling area	North	East	Level of protection	Gravel	Sand	Silt	TOC
Gorgona	Punta di Cala Scirocco	43°24.995′	09°53.942′	1	0.3	93.1	6.6	0.22
	Punta di Cala Maestra	43°26.300′	09°54.050′	2	4.6	93.6	1.8	0.48
Capraia	Punta del Fondo	43°02.389′	09°47.589′	1	2.8	61.5	35.7	0.28
Pianosa	Isolotto 'la Scarpa'	42°37.197′	10°05.665′	1	0.2	99.3	0.5	1.70
	Punta Secca	42°34.450′	10°06.600′	1	<0.1	99.4	0.6	0.34
Montecristo	Cala dello Scoglio	42°20.099′	10°17.330′	1	1.2	87.7	11.1	0.61
	Cala Grande	42°18.597′	10°19.011′	1	0.3	98.9	0.8	0.34
	Cala Maestra	42°20.047′	10°19.326′	1	27.8	65.8	6.4	0.54
	Cala del Santo	42°20.436′	10°19.273′	1	2.8	80.2	17.0	0.31
Giannutri	Grottoni	42°14.323′	11°06.351′	1	0.1	98.0	1.9	0.83
	Cala dello Scoglio	42°15.062′	11°06.434′	2	7.6	62.8	29.6	0.63

Notes: We performed $n = 4$ sampling replicates in each area, for each season. Average grain size was calculated based on a single island in the Tuscan Archipelago. The data are expressed as a percentage of the total dry weight. Gravel = particles of diameter > 2 mm; sand = particles of diameter between 2 mm and 0.63 μm; silt = percentage of particles of diameter < 63 μm. Total organic carbon (TOC) average composition was calculated based on a single island in the Tuscan Archipelago. Data are expressed as a percentage of the total dry weight.

The estuaries of the Arno, Cecina, Ombrone and Albegna Rivers are characterised by different flow rates (Table 2), and have discharge waters rich in nutrients and pollutants from inland agriculture and urban centres. The Arno River drains a wide inland area, transporting Al, Fe, Hg and other trace elements at high concentrations towards the sea [28]. Bibliographical data highlighted

Table 2. Flow rates for the main rivers.

River	Average flow rates
Cecina	$15\,m^3 \cdot s^{-1}$
Arno	$110\,m^3 \cdot s^{-1}$
Ombrone	$32\,m^3 \cdot s^{-1}$
Albegna	$15\,m^3 \cdot s^{-1}$
Magra	$40\,m^3 \cdot s^{-1}$

pollution from the Cecina River, due to inland industrial activity [29]. The coastal area of Livorno is characterised by the presence of Europe's largest chloride–alkali plant, which is responsible for marine Hg pollution [30]. Significant pollution due to the presence of both the Ombrone and Albegna effluents was recognised [31,32]. The central part of Tuscany is characterised by the cinnabar (HgS) deposits of Mount Amiata, an inactive volcano, where mining and smelting began during the Etruscan period (eighth to first centuries BCE) and stopped in 1980 [33]. High levels of trace elements were recorded in sediments from the island of Elba to Mount Argentario [34,35]. In addition, three large coastal towns, Pisa, Livorno and Cecina, located in the northern part of the study area, discharge partially treated effluent into rivers. The whole coastal area also experiences summer tourism, which leads to a substantial increase in inhabitants. As a consequence, municipal waste water treatment plants show effluents characterised by worsened water quality and an increase in the nutrient concentration of marine water [36].

2.2. *Sampling methodology*

Twelve stations were sampled from 2005 to 2008, with four campaigns performed per year, to take account of seasonal fluctuations. Four replicates were carried out at each sampling station, giving a total of $n = 48$ observations per year. Water samples for chemical and biological analyses were collected using a pre-cleaned Niskin bottle. Unfiltered aliquots for total nutrient analysis were collected in high-density polyethylene (HDPE) bottles, and a 3 L aliquot was filtered *in situ* using a cellulose acetate fibre filter disk (Millipore, 0.45 μm fibre diameter) for dissolved nutrient analysis, according to IRSA-CNR methods [37]. Water samples for chlorophyll-a analysis (Chl-a, $\mu g \cdot L^{-1}$) were filtered through a glass-whole fibre filter disk (GF/F, Millipore), according to the IRSA-CNR method [38] for marine oligotrophic waters, and filters were collected in Teflon vials and stored on ice in the dark. Water samples for microbiological analyses were collected in sterile bottles according to asepsis rules. Superficial sediment samples (0–10 cm) were collected in HPDE sterile bottles by scuba divers from sites with a bathymetry between 15 and 20 m. Sediment pH and redox potential (Eh, mV) were measured *in situ* using field probes (Crison pH 25 with a combined pH electrode, and Crison Eh 25 with Pt and Ag/AgCl reference electrodes). Codified water and sediment samples were stored in the dark and kept on ice at 4 °C during transportation to the laboratory, where they were processed immediately.

2.3. *Laboratory analyses*

2.3.1. *Water analyses*

Determination of the chemical and biological variables was performed by spectrophotometry, using a 6505 UV/Vis (Jenway) double-ray spectrophotometer. Dissolved nutrients (ammonia, nitrite, nitrate, soluble reactive phosphorus (SRP), total nitrogen (TN) and total phosphorus (TP)) were quantified following the methods of Parsons et al. [39] and Grasshoff et al. [40]. Dissolved inorganic nitrogen (DIN) was obtained mathematically as the sum of ammonia, nitrite and nitrate

and was used to calculate the ratio DIN/SRP. Chl-a was extracted using 90% acetone with the addition of two drops of magnesium carbonate suspension to aid retention and guard against the development of acidity in the filtrate [39,41]. Absorbances were measured before and after acidification of the sample with 20 μL HCl 18% (v/v). A six-points reference curve, obtained as a linear regression of the absorbances from scalar dilution of a standard reference solution (J.T. Baker, Chem B.V., Deventer, The Netherlands), was constructed on samples according to the internal addition procedure [42]. Average recovery percentages were within 95–101.1% and analytical concentrations were not recovery corrected. Limits of quantification (LOQ) were calculated for the adopted procedures by progressive serial dilution of a standard solution and were 0.1 μM for all determined nutrients and 0.01 $\mu g \cdot L^{-1}$ for Chl-a.

2.3.2. *Sediment analyses*

Grain size, total organic carbon (TOC) and POPs were determined in the sediment samples. Chemicals and reagents were of analytical grade and the glassware used was cleaned carefully to avoid sample contamination. Grain size was determined using a set of steel test sieves (DIN EN ISO 9001) of various diameters [43] and the Udden–Wentworth scale was used to classify sediment texture. TOC analyses were performed using a CHNS/O Analyzer (Perkin–Elmer) [44]. The extraction of PCBs, HCB, *p,p′-DDE*, PAHs and PBDE was performed on 10 g of air-dried sediments using a Soxhlet extractor with 250 mL dichloromethane (16 h), followed by purification on a silica and anhydrous sodium sulfate (Na_2SO_4) gel column activated at 120 °C for 24 h. The column was eluted with 32.5 mL of hexane (for PCBs, HCB, *p, p′*-DDE and PBDE) followed by 15 mL of a hexane/dichloromethane solution 1 : 1 v/v (for PAHs). PCB congeners were analysed by gas chromatography (Perkin–Elmer model Autosystem) with a ^{63}Ni electron capture detector (HRGC-ECD) and a Supelco SBP-5 capillary column (30 m long, 0.25 mm i.d., 0.25 μm film thickness). Sixteen PAHs considered priority pollutants by the US Environmental Protection Agency were identified using high-performance liquid chromatography (HPLC). Acenaphthylene was determined using a Waters PDA 996 photodiode series detector, whereas a Waters 474 scanning fluorescence detector was used for all the other PAHs. Chromatographic separation was performed on a Supelcosil™ LC-PAH HPLC chromatographic column (250 × 4.6 mm i.d., particle size 5 μm, Supelco). Detection limits were evaluated as mean blank +3 SD and ranged from 0.001 to 0.075 $ng \cdot g^{-1}$ dry weight for PCBs and 0.01 to 0.5 $ng \cdot g^{-1}$ dry weight for PAHs. A certified reference material, HS-6 harbour sediments, purchased from NRC, Canada, procedural blanks and replicate samples were used for the quality control procedures, and their reproducibility and recovery were high (70–80%). Nineteen BDE congeners (IUPAC numbers BDE3, BDE7, BDE5, BDE17, BDE28, BDE49, BDE71, BDE47, BDE66, BDE77, BDE100, BDE119, BDE99, BDE85, BDE126, BDE154, BDE153, BDE138, BDE156) were identified and quantified using an ion-trap mass spectrometer (GC/MS) from ThermoFinnigan (Trace GC 2000/GC Polaris), equipped with an AS2000 autosampler (Rtx-5MS capillary column, 30 m × 0.25 mm i.d., film thickness 0.25 μm; Restek). The internal standard used was PCB1413C (Cambridge Isotope Laboratories) and the compounds detected in the blanks were PBDEs 99 and 154. The LOQ value was 0.04 $pg \cdot g^{-1}$ dry weight. Results were reported as the total sum of single congeners for PCBs and PBDE. PAHs were expressed as the total sum, and molecular ratios were also reported.

2.3.3. *Determination of microbial indices*

To determine the heterotrophic bacteria, serial dilution in filtered marine water was prepared for each water sample. Aliquots of 100 μL of each dilution were collected and spread on the surface

of three Petri dishes containing a culture medium for heterotrophic bacteria (Marine Agar; Difco, Bologna) and incubated at 28 °C. Grown colonies were counted after 48 h and 1 week of incubation. The number of colonies for each water sample was reported as colony-forming units (CFU) $\cdot$ mL^{-1} of water sample. The presence of bacteria belonging to the *Actynomyces* was detected in the same way as above, but using *Actynomyces* Isolation Agar (Difco). Moreover, water samples were tested for the presence of hydrocarbon-degrading bacteria as bioindicators of the eventual presence of dispersed hydrocarbons. Aliquots of 100 μL of each dilution were collected and spread on the surface of Petri dishes containing mineral salt basal medium (MSBM; 1.5 g KH_2PO_4, 7.9 g $Na_2HPO_4 \times 2H_2O$, 0.8 g NH_4Cl, 0.1 g $MgSO_4 \times 7H_2O$ and 10 mL of Pfenning's trace element solution per litre of bidistilled water), in the presence of diesel fuel consisting mostly of aliphatic hydrocarbons, in particular paraffin (C_{12}–C_{28}), at a concentration of 2%. The composition of the trace element solution was: 0.1 g $ZnSO_4 \times 7H_2O$, 0.03 g $MnCl_2 \times 4H_2O$, 0.3 g H_3BO_3, 0.2 g $CoCl_2 \times 6H_2O$, 0.01 g $CuCl_2 \times 2H_2O$, 0.02 g $NiCl_2 \times 6H_2O$, 0.03 g $Na_2MoO_4 \times 2H_2O$ and 0.05 g $FeCl_3$. The solid culture medium for Petri dishes was obtained by adding 1.6% agar (Difco) and 50 μL of sterile diesel fuel, and this was spread on the surface of the agar before sample addition. Enrichment cultures were arranged in MSBM in the presence of 2% diesel fuel as the sole carbon and energy source, with an inoculum of 1 mL of the different water samples. After a period of 2–3 weeks, 100 μL aliquots were collected from cultures showing turbidity, as an indication of bacteria growth, and spread on the surface of Petri dishes containing solid MSBM and diesel fuel as the sole carbon and energy source. The Petri dishes were incubated for 1 week and colonies showing different characteristics in terms of margin shape, colour and aspect were chosen and streaked onto the surface of new Petri dishes containing MSBM plus diesel fuel and the complex medium YEPG, containing per litre of bidistilled water: 5 g tryptone (Difco, Bologna), 2.5 g D-glucose (BDH, Milan) and 2.5 g yeast extract (Oxoid, Milan). Bacterial strains were then streaked onto new Petri dishes for at least three successive passages, and 2 mL of cultures in liquid media were then transferred into cryovials (Nalgene, Milan) in the presence of 30% sterile glycerol and maintained at −80 °C. Cultures of hydrocarbon-degrading bacteria obtained from water samples were observed for growth and substrate transformation. Images of the cultures in MSBM and diesel fuel were taken using a Kodak model 01 camera. The details highlighted in the images were the presence of biofilm and adhesion of the bacterial cells to the hydrophobic substrate (diesel fuel). One hundred microlitres were harvested from enrichment cultures of hydrocarbon-degrading bacteria and 5 μL of an Acridine Orange solution (2000 μg $\cdot$ mL^{-1}; Agar, Milan) were added and samples were incubated for 10 min at room temperature. Samples were centrifuged at 6000 g, and washed twice with phosphate-buffered saline at pH 7.2. Aliquots of the treated sample (30 μL) were placed on a slide and observed by fluorescence microscopy (Zeiss, model 3067), with emission wavelengths of 450 and 420 nm. Images were collected by using a Kodak (model 01) camera.

2.4. *Statistical analyses*

Statistical analyses were performed using unvariate and multivariate methods. Mean, standard deviation, minimum and maximum values, linear regressions and Spearman's rank correlation matrices ($n = 192$) were performed using Statistica 7.0 (StatSoft Inc., Padova). Correlations with probability values of $p < 0.01$ were considered significant (**). Multivariate analyses were developed using Primer-E v. 6.0 software (Plymouth Marine Laboratory, Plymouth, UK). Principal component analysis (PCA) and nonmetric multidimensional scaling (nmMDS) were applied to the data to explore similarities and dissimilarities among variables, following the procedures reported in Renzi et al. [25]. Significant differences by 'years', 'island' and 'level of protection'

were tested using analysis of similarities (ANOSIM R statistic; one-way); pairwise testing was also used to highlight significant system differences.

3. Results

3.1. *Water characterisation*

Water physicochemical and biological means (range in parentheses) obtained on a yearly basis for each island are summarised in Table 3. Observed ranges for each variable were caused by typical seasonal fluctuations. NO_2^- values were always below detection limits during the observation period. Higher means for NH_4^+ were recorded in Capraia Island, whereas lower values were observed in Montecristo. Gorgona showed wider variability between years, ranging from LOQ

Table 3. Nutrient averages (range) on annual basis ($n = 16$) in superficial water of the Tuscan Archipelago from 2005 to 2008.

Variable	Unit	Year	Gorgona	Capraia	Pianosa	Montecristo	Giannutri
NH_4^+	μM	2005	1.3 (0.4–1.9)	1.5 (1.3–1.6)	0.6 (<0.1–0.8)	0.2 (<0.1–0.2)	0.5 (<0.1–0.7)
		2006	0.1 (<0.1–0.1)	1.1 (0.9–1.4)	0.2 (<0.1–0.2)	0.3 (<0.1–0.4)	0.6 (0.4–1.2)
		2007	1.7 (0.8–2.1)	1.2 (0.8–1.4)	0.8 (0.5–0.9)	0.4 (<0.1–0.5)	0.6 (0.3–0.8)
		2008	0.7 (0.4–0.9)	1.0 (0.9–1.1)	1.0 (0.7–1.3)	0.2 (<0.1–0.3)	0.7 (0.6–0.9)
NO_2^-	μM	2005	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)
		2006	0.1 (<0.1–0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)
		2007	<0.1 (<0.1)	<0.1 (<0.1)	0.1 (<0.1–0.1)	0.2 (0.1–0.2)	<0.1 (<0.1)
		2008	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)
NO_3^-	μM	2005	2.0 (1.8–2.4)	2.6 (2.3–2.8)	4.4 (4.2–4.7)	7.3 (6.5–7.9)	5.5 (4.9–6.3)
		2006	1.1 (0.9–1.5)	2.3 (2.1–2.5)	3.6 (3.0–4.1)	8.3 (8.0–8.6)	3.1 (1.9–3.5)
		2007	1.0 (0.9–1.3)	2.8 (2.7–2.9)	2.9 (2.7–3.1)	8.1 (7.6–8.5)	2.9 (2.1–3.5)
		2008	1.5 (1.3–1.7)	2.7 (2.0–2.9)	3.5 (3.1–3.9)	6.7 (6.4–7.3)	1.2 (0.5–1.6)
DIN	μM	2005	2.4 (1.7–2.9)	4.0 (2.8–5.2)	5.0 (4.4–5.3)	7.5 (6.3–8.4)	6.0 (5.1–7.4)
		2006	1.0 (0.6–1.5)	3.4 (2.1–3.6)	3.7 (2.7–4.1)	8.6 (5.7–9.7)	3.7 (3.2–5.1)
		2007	2.7 (2.3–2.9)	4.1 (3.5–4.6)	3.8 (2.9–4.2)	8.4 (7.2–9.1)	3.5 (2.9–5.3)
		2008	2.2 (1.5–2.7)	3.7 (2.9–3.9)	4.5 (3.6–4.9)	6.9 (5.9–7.9)	1.9 (1.1–2.1)
TN	μM	2005	6.7 (5.6–8.2)	10.0 (8.5–11.2)	27.6 (21.4–32.6)	32.2 (27.4–34.7)	19.9 (16.7–21)
		2006	7.2 (6.8–7.9)	10.3 (8.9–12.3)	24.6 (22.8–29.5)	43.2 (39.5–47.9)	11.0 (9.1–13.6)
		2007	7.8 (6.7–8.2)	7.8 (6.9–9.5)	37.0 (31.5–40.1)	47.8 (41.6–51.2)	13.5 (9.8–14.6)
		2008	5.6 (4.8–7.3)	7.4 (6.8–8.3)	29.3 (22.4–31.6)	13.8 (11.5–15.6)	11.0 (8.3–12.5)
SRP	μM	2005	0.1 (<0.1–0.2)	0.1 (<0.1–0.1)	0.2 (<0.1–0.3)	0.2 (0.1–0.3)	0.1 (<0.1–0.1)
		2006	0.3 (0.1–0.4)	<0.1 (<0.1)	0.2 (<0.1–0.3)	0.1 (<0.1–0.1)	0.1 (<0.1–0.1)
		2007	<0.1 (<0.1)	<0.1 (<0.1)	0.2 (<0.1–0.3)	0.2 (<0.1–0.3)	<0.1 (<0.1)
		2008	0.1 (<0.1–0.1)	<0.1 (<0.1)	0.1 (<0.1–0.1)	<0.1 (<0.1)	<0.1 (<0.1)
TP	μM	2005	0.1 (<0.1–0.1)	0.5 (0.1–0.6)	0.4 (<0.1–0.7)	0.5 (0.2–0.6)	0.2 (<0.1–0.3)
		2006	0.2 (<0.1–0.3)	0.5 (<0.1–0.6)	0.3 (<0.1–0.5)	0.3 (<0.1–0.4)	0.3 (<0.1–0.4)
		2007	0.1 (<0.1–0.1)	0.4 (<0.1–0.6)	0.3 (<0.1–0.4)	0.3 (0.1–0.4)	0.1 (<0.1–0.1)
		2008	0.1 (<0.1–0.2)	0.4 (0.1–0.5)	0.4 (<0.1–0.6)	0.9 (0.5–1.0)	0.2 (0.1–0.3)
DIN/SRP		2005	24 (22–36)	45 (37–49)	26 (21–33)	44 (36–49)	60 (58–62)
		2006	nc	nc	19 (16–22)	78 (61–83)	33 (26–39)
		2007	nc	nc	25 (22–36)	56 (41–61)	nc
		2008	22 (11–25)	nc	74 (69–79)	nc	nc
Chla	$\mu g \cdot L^{-1}$	2005	0.5 (0.1–0.6)	0.2 (<0.1–0.3)	0.2 (<0.1–0.3)	0.2 (<0.1–0.2)	0.4 (0.2–0.6)
		2006	0.4 (0.2–0.5)	0.2 (<0.1–0.2)	0.3 (0.1–0.4)	0.4 (0.1–0.5)	1.0 (0.9–1.4)
		2007	0.4 (0.1–0.6)	0.4 (<0.1–0.5)	0.3 (0.2–0.4)	0.3 (<0.1–0.4)	1.5 (1.1–1.7)
		2008	0.2 (<0.1–0.3)	0.4 (0.1–0.4)	0.4 (0.1–0.5)	1.5 (1.2–1.7)	1.3 (1.0–1.5)

Notes: NH_4^+, ammonium; NO_2^-, nitrite; NO_3^-, nitrate; DIN, dissolved inorganic nitrogen; TN, total nitrogen; SRP, soluble reactive phosphorous; TP, total phosphorous; Chl-a, chlorophyll-a; nc, not calculable.

to 1.7 μM. NO_3^- was the most representative form of inorganic nitrogen, with the highest values recorded in Montecristo. Most of the total nitrogen was formed by the sum of dissolved organic nitrogen and particulate forms. In fact, TN levels were 2–7 times the DIN values. Both forms of phosphorous showed low levels, and SRP means were < LOQ in six of twenty occassions. Furthermore, the dominant form of TP was represented by SRP, whose mean values were half those of TP for all the observation periods. Higher primary productivity, explained by Chl-a, was observed in both Montecristo and Giannutri. DIN/SRP mean ratios were always >19, with higher values recorded in Montecristo and Pianosa. Spearman's rank correlations showed strong relationships between the following pairs: NH_4^+–NO_3^- (-0.82^{**}), NH_4^+–DIN (-0.84^{**}), NH_4^+–TN (-0.72^{**}), NH_4^+–SRP (-0.68^{**}), DIN–Chl-a (-0.57^{**}) and SRP–TN (-0.97^{**}).

Microbiological analyses of samples were conducted using a culture approach and gave information on the presence of different metabolic groups of bacteria. The heterotrophic bacteria population showed constant values ranging from 10^3 to 10^4 CFU · mL^{-1} for the study period (Figure 2). Filamentous bacteria belonging to the *Actinomycetes* group and hydrocarbon-degrading bacteria were tested in the same water samples as bio-indicators of anthropogenic impact. Low concentrations of these bacterial groups were observed in all sites, but higher values of 10^2 CFU · mL^{-1} were found in the Montecristo reserve (Figure 3). Several types of hydrocarbon-degrading bacteria were isolated from water samples, and a study of their growth in the presence of diesel as the only carbon and energy source showed consumption of the hydrophobic substrate (Figure 4). The adhesion of hydrocarbon-degrading bacteria to hydrophobic substrates was documented in sample duplicates B and C, when compared with control A, without bacteria added (Figure 5). These results confirmed the presence of hydrocarbon-degrading bacteria in water samples from the islands of the Tuscan Archipelago, suggesting the presence of hydrocarbon sources in the marine reserve.

Multivariate statistical analyses were performed on this data set to evaluate any similarity between islands in terms of water characteristics and significant trends on a yearly basis. Applying PCA analysis, two principal components (PC1 57.8% and PC2 23.1%) were obtained, accounting

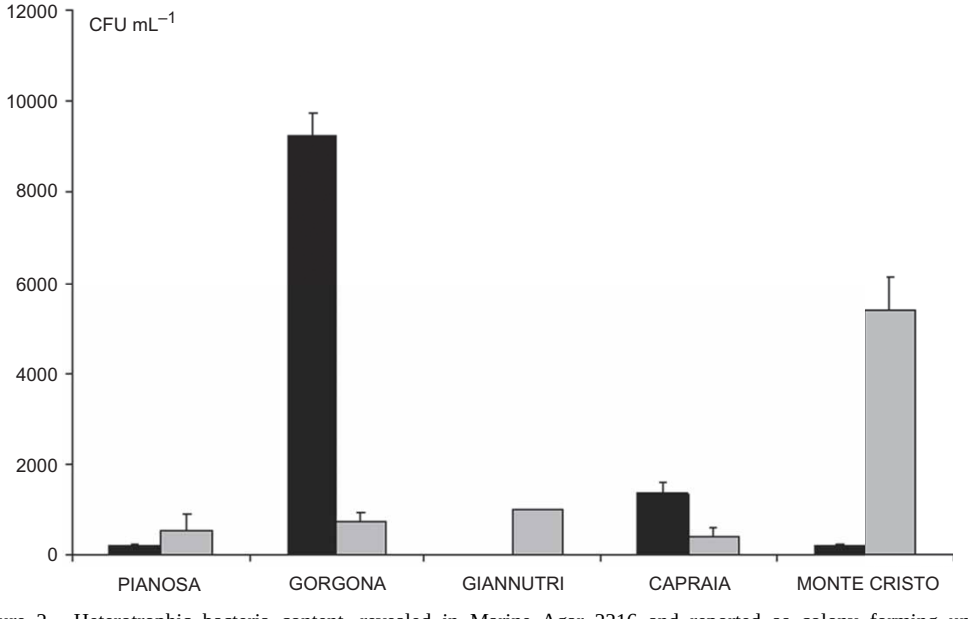

Figure 2. Heterotrophic bacteria content, revealed in Marine Agar 2216 and reported as colony forming units (CFU) · mL^{-1} in water samples collected from the marine reserve area. For each island, black bars report levels measured in 2007, whereas grey bars report 2008 levels.

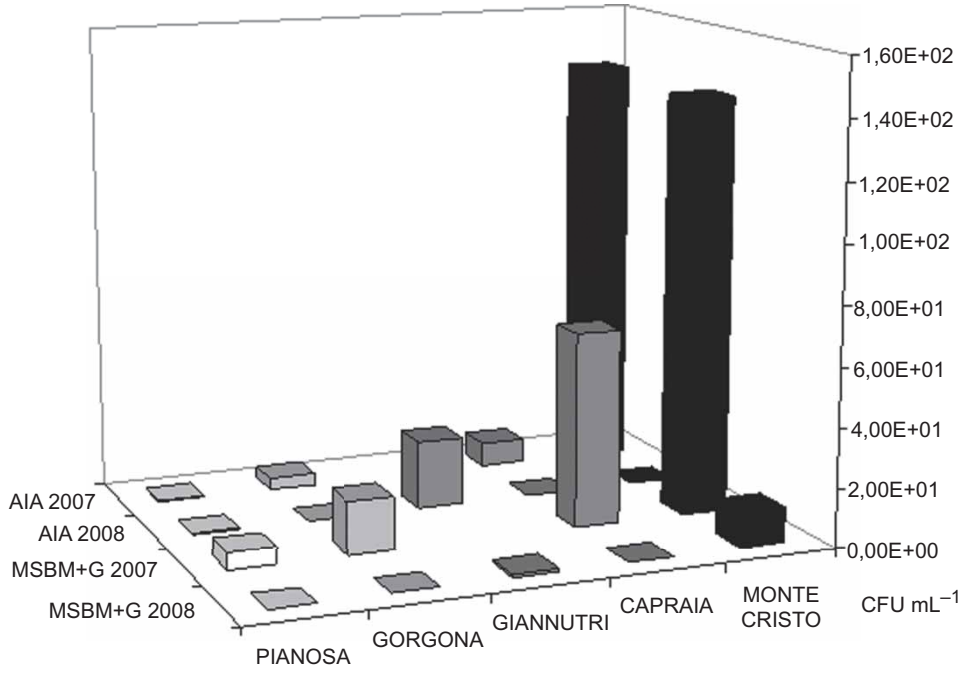

Figure 3. Concentrations of *Actinomycetes* (AIA 2007 and AIA 2008) and hydrocarbon-degrading bacteria (MSBM-G2007 and MSBM-G2008) in water samples collected from the marine reserve area. Bacteria were grown in *Actinomycetes* Isolation Agar (AIA) and solid MSBM plus 2% diesel fuel, and are reported as colony forming units (CFU) $\cdot$ mL^{-1}. Values on the y-axis are reported in scientific E notation.

for 78.9% of the total variance. Analysis of the coefficients in linear combinations of variables making up the principal components showed that PC1 was significantly related to NH_4^+ (0.419), Chl-a (0.382), SRP (-0.428), TN (-0.447), DIN (-0.423) and NO_3^- (-0.319). PC2 showed the highest positive correlations with SRP (0.216) and the highest negative correlations with NO_3^-

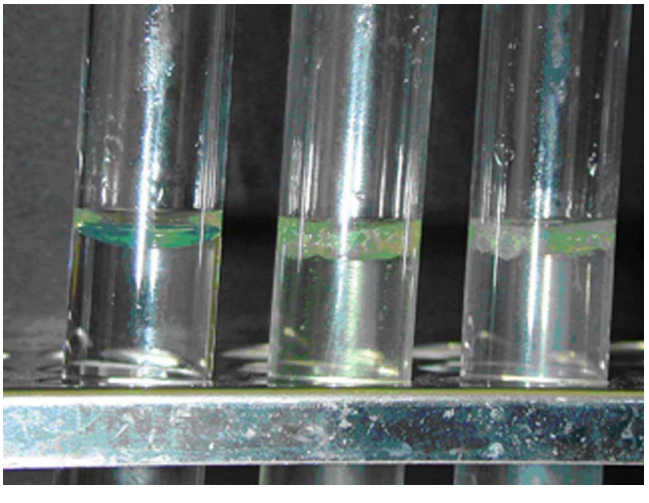

Figure 4. Growth of hydrocarbon-degrading bacteria in liquid MSBM plus 2% diesel fuel, showing the bacterial biomass and the use of hydrocarbons still present in the control without bacteria added.

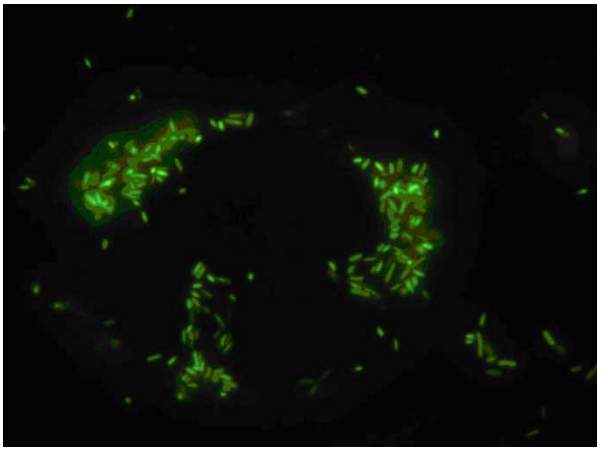

Figure 5. Fluorescence microscopy image showing bacterial cells adhering to hydrocarbon. Bacterial cells were highlighted using the probe Acridine Orange.

(−0.469), TP (−0.656) and DIN/SRP (−0.427). The ordination diagram is shown in Figure 6, where cluster overlay is also reported. Capraia, Gorgona and Giannutri are well-segregated from Pianosa and Montecristo, which showed opposite distributions (along PC2) in relation to nutrient and microbiological assessment.

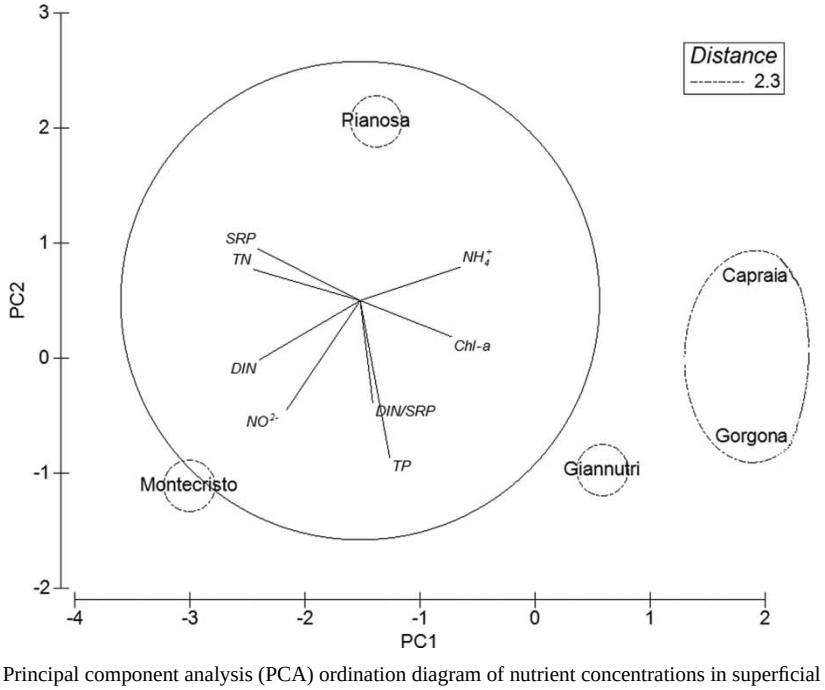

Figure 6. Principal component analysis (PCA) ordination diagram of nutrient concentrations in superficial water from the Tuscan Archipelago. The first two components (PC1 and PC2) together accounted for 80.8% of the total variance. NH_4^+, ammonium; NO_2^-, nitrite; NO_3^-, nitrate; DIN, dissolved inorganic nitrogen; TN, total nitrogen, SRP, soluble reactive phosphorous; TP, total phosphorous; Chl-a, chlorophyll-a. Arrows (both slope and length) represent the correlations between sediment variables and the principal axes PC1 and PC2. Correlation coefficients are given in the text.

Table 4. Total average content (range) of persistent organic pollutants (POPs) measured on an annual basis ($n = 16$) in superficial sediments of the Tuscan Archipelago from 2005 to 2008.

Variable	Year	Gorgona	Capraia	Pianosa	Montecristo	Giannutri
ΣPCBs ($ng \cdot g^{-1}$)	2005	0.15 (0.11–0.19)	0.36 (0.22–0.49)	0.30 (0.10–0.93)	0.67 (0.41–0.72)	0.16 (0.11–0.23)
	2006	1.11 (0.98–1.23)	1.28 (1.17–1.41)	0.43 (0.36–0.53)	0.53 (0.49–0.67)	6.14 (4.96–7.22)
	2007	1.33 (1.12–1.36)	1.41 (1.11–1.50)	1.11 (1.08–1.22)	1.37 (1.12–1.46)	0.82 (0.75–1.12)
	2008	0.99 (0.72–1.14)	5.22 (4.08–5.39)	1.56 (1.41–1.63)	0.81 (0.59–0.92)	1.12 (0.96–1.22)
HCB ($ng \cdot g^{-1}$)	2005	1.54 (1.33–1.64)	0.17 (0.09–0.26)	0.01 (<0.01–0.01)	0.39 (0.17–0.49)	0.20 (0.14–0.36)
	2006	0.45 (0.22–0.79)	0.24 (0.18–0.31)	0.29 (0.09–0.31)	0.26 (0.11–0.37)	0.05 (<0.01–0.09)
	2007	0.20 (0.09–0.36)	0.06 (<0.01–0.13)	0.08 (<0.01–0.11)	0.16 (0.09–0.26)	0.07 (<0.01–0.14)
	2008	0.44 (0.16–0.54)	0.10 (<0.01–0.18)	0.34 (0.17–0.41)	0.43 (0.35–0.53)	0.01 (<0.01–0.07)
p,p'–DDE ($ng \cdot g^{-1}$)	2005	0.43 (0.27–0.47)	0.18 (0.05–0.20)	0.24 (0.12–0.33)	0.22 (0.07–0.32)	0.23 (0.16–0.36)
	2006	0.31 (0.29–0.34)	0.14 (0.08–0.19)	0.11 (0.01–0.16)	0.14 (0.05–0.23)	0.20 (0.09–0.32)
	2007	0.21 (0.12–0.43)	0.08 (<0.01–0.15)	0.08 (<0.01–0.13)	0.22 (0.09–0.32)	1.52 (1.33–1.65)
	2008	0.37 (0.20–0.41)	0.56 (0.38–0.67)	0.36 (0.17–0.46)	0.65 (0.51–0.72)	0.07 (<0.01–0.09)
ΣPAHs ($ng \cdot g^{-1}$)	2005	2.45 (2.12–2.59)	1.57 (1.39–1.62)	2.20 (2.05–2.36)	2.29 (2.12–2.45)	1.66 (1.15–1.71)
	2006	1.24 (1.19–1.33)	3.20 (2.98–3.41)	3.17 (2.87–3.22)	2.25 (2.16–2.33)	1.82 (1.79–1.96)
	2007	0.50 (0.16–0.62)	1.95 (1.87–2.08)	1.45 (1.39–1.59)	3.46 (3.09–3.56)	7.85 (7.36–7.92)
	2008	5.95 (5.62–5.99)	4.20 (4.13–4.36)	5.00 (4.67–5.23)	1.55 (1.19–1.72)	3.70 (2.93–4.22)
ΣPBDE ($pg \cdot g^{-1}$)	2005	24.52 (24.11–25.33)	16.41 (13.27–17.36)	9.53 (8.33–9.46)	11.79 (10.06–13.02)	4.88 (4.65–4.59)
	2006	17.17 (10.91–19.26)	12.71 (8.43–14.92)	12.51 (11.02–13.21)	13.84 (11.00–15.27)	6.08 (6.02–6.32)
	2007	11.17 (9.33–15.82)	11.15 (8.74–13.26)	14.34 (13.24–15.03)	10.92 (9.33–12.45)	5.10 (4.95–5.38)
	2008	23.19 (17.83–26.43)	<0.01 (<0.01)	6.27 (5.61–6.43)	14.72 (13.21–15.76)	8.45 (7.52–8.83)

Notes: ΣPCBs, polychlorinated biphenyl; HCB, hexachlorobenzene; p,p'-DDE, 1,1-*bis*-(4-chlorophenyl)-2,2-dichloroethene; ΣPAH, polycyclic aromatic hydrocarbons; ΣPBDE, polybrominated diphenylethers. Values are expressed as dry weight.

3.2. *Sediment characterisation*

Collected sediments, mostly characterised by calcareous detritus of biological origin, showed pH and Eh values typical of well-oxygenated sediments, for all monitored sites, with Eh values never lower than 125 mV. Grain-size composition (gravel, sand and silt) is reported in Table 1. A higher silt content was observed in Capraia, while Pianosa sediments mainly comprised sands and calcareous detritus. Montecristo and Giannutri showed the same percentage of dimensional classes. Regarding TOC, observed values were <1%, except for Pianosa, where maximum TOC values of 2.0% dry weight were observed. Very low standard deviations were observed, highlighting a general homogeneity in TOC distribution, except for Pianosa, where wide fluctuations were recorded. In relation to POPs, mean values (range in parentheses) calculated on a yearly basis for each island are summarised in Table 4. For PCBs, identified congeners were represented by highly chlorinated PCBs (i.e. from esa- to octachlorobiphenils) and a nonsignificant temporal trend for ΣPCB was observed. Organochlorinated pesticide levels were within the range < LOQ to 1.54 ng · g^{-1} dry weight for HCB and 0.07 to 0.65 ng · g^{-1} dry weight for p,p'-DDE. For Σ16 PAHs levels, the highest values were measured in sediments from Gorgona and Giannutri, while Montecristo (Cala del Santo, Cala Maestra and Cala Corfù) showed a trend for decreasing ΣPAHs over the years. The relative percentage of the 16 US Environmental Protection Agency PAHs, calculated with respect to the total PAHs for each island, are reported in Table 5. Flu (26%), Py (14%), BaA (11%) and Chry (11%) represent over 60% of the total amount of measured PAHs. PAH levels may be produced from both pyrolytic (incomplete combustion processes at very high temperature) and petrogenic (petroleum products) sources [45,46]. The use of PAH ratios is a well-known method for interpreting the composition of PAHs and establishing their origin [47]. The ratio between low molecular mass PAHs (L-PAH; NaP + AceP + A + Fl + Phe + An) and high molecular mass PAHs (H-PAH; Flu + Py + BaA + Chry + BbF + BkF + BaP + DBA + BghiP + IP) was calculated to assess the origin of the PAHs in sediments [48]. Low values for the L-PAHs/H-PAHs ratio were obtained during the study, and highlighted a predominantly pyrolytic origin for these contaminants. In Montecristo, a mixed pyrolytic–petrogenic origin was obtained. ΣPBDE was low and ranged from LOQ to 25.3 pg · g^{-1}, indicating a relatively unpolluted environment. Draftman's plot obtained for POPs is reported in Figure 7. A linear relationship between p,p'-DDE and HCB

Table 5. Relative percentage of the 16 US Environmental Protection Agency polycyclic aromatic hydrocarbons, calculated with respect to the total average for each island.

	Gorgona	Capraia	Pianosa	Montecristo	Giannutri
Chry	12	5	8	6	1
BbF	9	16	0	3	1
BkF	5	5	0	3	8
BaP	11	12	0	14	1
DBA	0	0	49	5	0
BghiP	4	6	0	9	5
IP	7	10	0	3	4
NaP	0	1	0	16	2
AceP	0	0	0	0	1
A	1	11	0	0	2
F	0	2	11	0	8
Phe	10	8	32	3	18
An	3	4	0	1	14
Flu	22	10	0	11	11
Py	6	5	0	17	15
BaA	10	5	0	9	9

Notes: Chry, chrysene; BbF, benzo[b]fluoranthene; BkF, benzo[k]fluoranthene; BaP, benzo[a]pyrene; DBA, dibenz[a,h]anthracene; BghiP, benzo(g,h,i)fluoranthene; IP, indeno[1,2,3-cd]pyrene; NaP, naphthalene; AceP, acenaphthylene; A, acenaphthene; F, fluorene; Phe, phenanthrene; An, anthracene; Flu, fluoranthene; Py, pyrene; BaA, benz[a]anthracene.

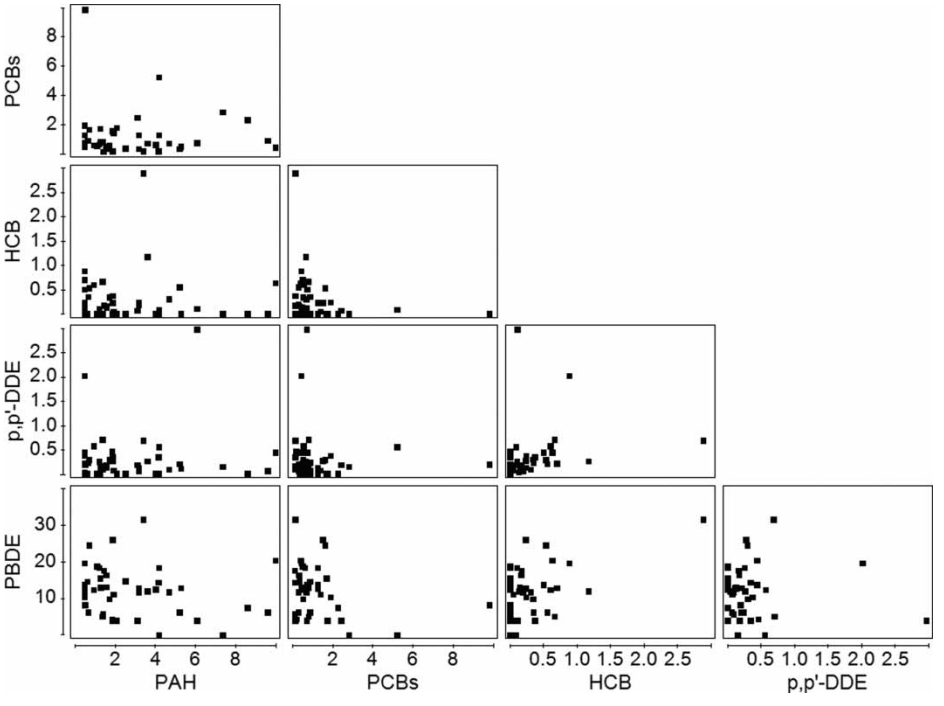

Figure 7. Draftman's plot relating to persistent organic pollutant (POP) values in sediments. ΣPCBs, polychlorinated biphenyl; HCB, hexachlorobenzene; p,p'-DDE, 1,1-*bis*-(4-chlorophenyl)-2,2-dichloroethene; ΣPAH, polycyclic aromatic hydrocarbons; ΣPBDE, polybrominated diphenylethers. The axes labels represent POP concentrations (in $ng \cdot g^{-1}$ dry weight).

was observed, although no relationship among PBDE and other pollutants was recorded, suggesting different sources and dynamics of transport. The higher silt content in Pianosa sediments was not statistically related to higher POP levels in this island. PCA performed on pretreated POP variables showed that the first two components (PC1 37.1% and PC2 27.0%) accounted for 64.1% of the total variance. In Figure 8 the loading plot is reported, showing that PC1 was mainly

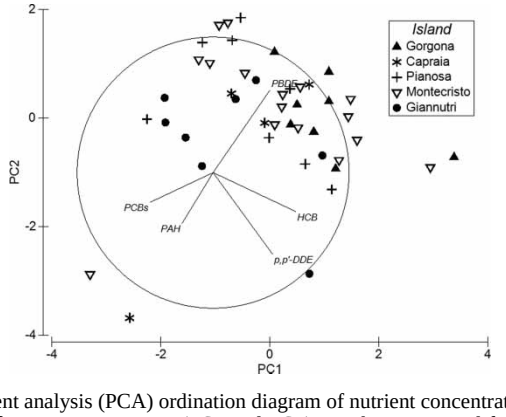

Figure 8. Principal component analysis (PCA) ordination diagram of nutrient concentrations in superficial water from the Tuscan Archipelago. The first two components (PC1 and PC2) together accounted for 80.8% of the total variance. ΣPCBs, polychlorinated biphenyl; HCB, hexachlorobenzene; p,p'-DDE, 1,1-*bis*-(4-chlorophenyl)-2,2-dichloroethene; ΣPAH, polycyclic aromatic hydrocarbons; ΣPBDE, polybrominated diphenylethers. Arrows (both slope and length) represent the correlations between sediment variables and the principal axes PC1 and PC2. Correlation coefficients are given in the text.

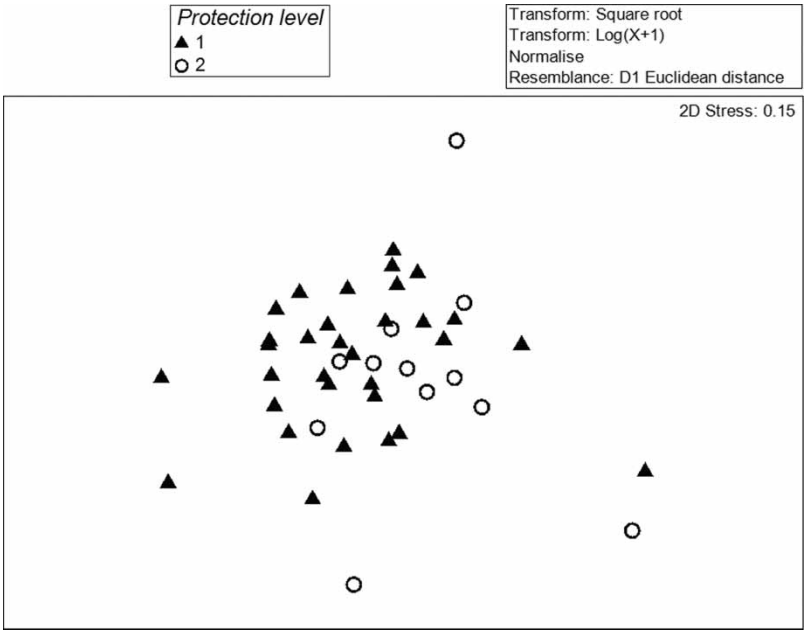

Figure 9. Non-metric multidimensional scaling (nmMDS) performed on persistent organic pollutant (POP) concentrations in sediments, considering the level of protection adopted in each island of the marine reserve as the discriminating factor for segregation. The symbols used (circles and triangles) represent the different level of protection: triangles, level 1; circles, level 2.

related to HCB (0.609), p,p'-DDE (0.437) and ΣPCBs (-0.462), whereas PC2 was mainly related to PBDE (0.607) and p,p'-DDE (-0.602). Similarities and dissimilarities among sites were explored by applying the ANOSIM test one-way and considering 'year' and 'island' as factors for significant differences. The results produced global R values of 0.015 (significance level of sample statistic = 28.4%) and 0.029 (significance level of sample statistic = 29.8%), respectively. Although no significant difference was observed testing the factors 'year' and 'island', pairwise testing showed highly significant differences between the following pairs: 2004–2006 (7.3 level %) and 2004–2007 (5.7 level %), Gorgona–Pianosa (8.2 level %), Gorgona–Giannutri (0.2 level %) and Montecristo–Giannutri (6.2 level %). By contrast, when testing the factor 'level of protection', the ANOSIM test produced a global R of 0.117 (significance level of sample statistic = 9.8%), indicating a significant difference in the distribution of POPs between the levels of protection 1 and 2. nmMDS performed on POP data according to the factor 'level of protection' is reported in Figure 9.

4. Discussion

European guidelines propose criteria for coastal water classification based on assessment of the trophic state and primary productivity indicators (Chl-a) [49]. In particular, the Water Framework Directive defines the nutrient levels representative of oligo-mesotrophic conditions ($NH_4^+ < 2\,\mu M$, $NO_3^- < 25\,\mu M$, TP $< 0.32\,\mu M$, Chl-a $< 8\,\mu g \cdot L^{-1}$), whereas mesotrophy is defined for Chl-a values ranging from 8 to $25\,8\,\mu g \cdot L^{-1}$ and TP levels within 0.32–1.13 μM. Nevertheless, single parameters often produce an assessment of the trophic level that is not well-defined. For this reason, many indices have been developed to evaluate relationships among nutrients [50]. An important index is the DIN/SRP ratio, which is indicative of nutrient limitation

for phytoplankton growth [51]. Previous studies have suggested that the optimal nutrient molar ratios for algal productivity are 16N:1P [52] and 31N:1P [53]. On the basis of these classification criteria, the results obtained in the Tuscan Archipelago highlight oligo-mesotropic conditions. In detail, Capraia, Montecristo and Giannutri were mainly characterised by P-limiting factors for phytoplankton growth, whereas in Gorgona and Pianosa the DIN/SRP ratio showed considerable variations from >20 to $\leqslant 16$ in 2008 (Gorgona) and 2006 (Pianosa), indicating nitrogen-limiting conditions for phytoplankton biomass. The absence of local pollution sources, such as wastewater effluent discharges, was also confirmed by the microbiology results, which highlighted low levels of strain caused by human activities. In marine systems, microorganisms play an important role in the recycling and distribution of organic matter [54]. In particular, heterotrophic bacteria are recognised as an important component of the planktonic community and contribute significantly to the regulation of the flux of organic matter. They are a critical link in the microbial loop, starting with the production of dissolved organic matter and ending with oxidation to CO_2 [55]. An indirect measure of the anthropogenic impact in these areas could be obtained by measuring *Actinomycetes* and hydrocarbon-degrading bacteria as bio-indicators of depuration-plant contamination and the dispersion of hydrocarbons by human activity, respectively [55,56]. The results obtained in water samples collected from the protected marine reserve pointed to a relative abundance of heterotrophic bacteria, related to the biogeochemical cycles [55], and a low level of bacteria as bio-indicators of environmental contamination, suggesting good conditions in the reserves. In addition, observed values were similar to those reported in areas where low contamination due to occasional human activity was detected [56]. Even at low levels, the presence of hydrocarbon-degrading bacteria indicated their ability to use a low bioavailability of carbon source and gain energy and carbon from hydrocarbons, removing these substrates from the environment [57,58]. Toxic substances tend to rapidly adsorb to sediment particles through chemical and physical complex mechanisms [59]. In particular, PAHs from pyrolytic processes are more strongly associated with sediments where organic matter has a key role, and are much more resistant to microbial degradation than PAHs of petrogenic origin [60]. Most classical POPs (e.g. PCBs, HCB, p,p'-DDE) can be classified as multi-hoppers, and for these molecules the contemporary environmental burden reflects both past and current primary emissions [61]. Even if some POPs, such as HCB, showed levels and diffusion controlled by environmental re-emissions, PAH dynamics were presumably controlled by current primary emissions. Because of this, and the occurrence of natural metabolisation [57], sediment PAH levels give information about the presence of local and recent sources of pollution. PAH levels were significantly lower than those found previously from the Moroccan Mediterranean coast (11–551 $\text{ng} \cdot \text{g}^{-1}$) [62], the north-west Mediterranean off the French coast (range in industrialised areas is 45–13,000 $\text{ng} \cdot \text{g}^{-1}$, the sum of 18 parent PAHs) [63] and the Rhone River estuary (between 1070 and 6500 $\text{ng} \cdot \text{g}^{-1}$, the sum of 11 PAHs) [64]. PCB levels were low compared with those reported by other authors for unpolluted marine areas in the Mediterranean Sea [45] and similar to those reported from superficial sediments from the Mediterranean between the Balearic Islands and Corsica [65] and with background levels established in the literature [66]. PBDE values for Tyrrhenian sediments have not been previously reported in the literature, and for this reason, our results represent a baseline for the definition of pollution levels in the central Tyrrhenian Sea. POP values measured in this study are probably related to the long-range atmospheric transport of multihopper molecules and the lower range transport of PAHs from both coastal pyrolitic and local petrogenic sources. Despite the low PAH levels observed, a clear difference due to the petrogenic and pyrolitic fingerprint was highlighted. These findings were also confirmed by the presence of hydrocarbon-degrading bacteria isolated at higher levels in Montecristo, which also showed significant statistical differences related to POP levels compared with the other islands. The observed trend of decreasing PAH levels from 2005 to 2008 in sediments from Montecristo could be related to the occurrence of an auto-purification process from previous petrogenic pollution, driven by microbiological activity.

In these systems, the carbon cycle plays a key role in the fate of POPs; marine-settling fluxes and biodegradation are examples of POP sinks driven by phytoplankton dynamics and the bacterial loop, respectively [61]. The results obtained in this study highlight that these areas do not protect marine quality against major threats from indirect chemical pollution. Although there were significant differences in the determined level of protection among islands, low but significant PAH contamination was observed in integral reserves (no-take, no-access zones), indicating that there are several threats against which marine systems cannot provide any direct protection. In spite of their clear ecotoxicological relevance to marine biota, these chemicals have rarely been considered by standard monitoring programmes in Marine Protected Areas. This *a priori* exclusion is a notable mistake. The effects of long-range run-off depositions, coastal human activity, tourism and fishing are elements that have to be considered not only for their ecotoxicological implications, but also to evaluate general local dynamics between coastal and marine systems and their effects on protected areas. The most important reason for the limited effectiveness of marine reserves is that the scale of processes in marine systems is often much greater than that encompassed by the reserve. Thus, reserves, although a critical component of any conservation strategy, must be coupled with other, complementary measures. The effectiveness of reserves, for example, may benefit greatly from a better understanding of the sources, fate and impact of chemicals in the sea. Critical to this is the ability to predict the likelihood and magnitude of contamination originating from external sources inside the reservoirs. These forecasts should be based on accurate estimates on the origin, dispersal and longevity of contaminants, as well as on the response of reserve inhabitants to potential levels of contamination. Providing guidelines for more comprehensive strategies for marine environment protection could represent a strategy for protecting populations against pollution sources located both within the marine reserve's boundaries and also outside.

5. Conclusions

A clear, general high quality was observed in relation to both water and sediment for the Tuscan Archipelago marine reserves. Nevertheless, the factor 'level of protection' allows significant differences among islands. Low, but significant, hydrocarbon contamination of petrogenic origin was observed in Montecristo Island (a no-take, no-access zone). These data confirm that there are several threats against which marine systems cannot be protected. This study could be a starting point for more thorough analyses of conservation strategies. Future efforts to manage and protect these ecosystems should take into account the origin and dispersal of contaminants, and provide guidelines for comprehensive strategies.

Acknowledgements

The authors are grateful to the National Park of the Tuscan Archipelago for supporting this research. Particular acknowledgement goes to the Scuba Divers' Association (FIPSAS, Siena) for their sampling activities and to CoNiSMa for supporting this activity by furnishing the *Universitatis* research boat.

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