



# Long-term changes of the trophic status in transitional ecosystems of the northern Adriatic Sea, key parameters and future expectations: The lagoon of Venice as a study case

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#### **Abstract**

The determination of the trophic status of transitional ecosystems from the physico-chemical and biological point of view is one of the requirements of the European Water Framework Directive (WFD 2000/60/EC). In Italy, its determination is implemented by the Regional Agencies for Environmental Protection (ARPAs) that have activated multi-annual monitoring programs. However, as the availability of funds is increasingly scarce, the number of environmental parameters to detect environmental changes should be conveniently managed.

The high number of environmental parameters, nutrient and macrophyte datasets available for the LTER-Italia site "Venice lagoon" can be an useful tool to analyze the trophic changes over recent years and to foresee environmental evolutions. Nutrient data on a spatial basis have been available since 1948, whereas macroalgal maps date back to 1980. The aim of this paper is to highlight the changes of the trophic status of the lagoon since the middle of the 20th century by considering the concentrations of nutrients in the surface sediments and in the water column, the variation of some physico-chemical parameters and the biomass of macroalgae and also to foresee the way it will possibly evolve. In fact, after many anthropogenic impacts that in the second half of the 20th century affected the lagoon, starting from

the year 2010, the ecological status is progressively improving. Nutrients show a significant reduction both in the water column and in surface sediments, and the macrophytes are represented by species of higher ecological value while the opportunistic species such as the Ulvaceae are in strong regression.

## **Keywords**

LTER-Italy network, trophic status, nutrient concentrations, waters, sediments, key parameters

## Introduction

Almost half of Europe's population lives less than 50 kilometers from the sea and the resources of the coastal areas and transitional water systems (TWS) produce much of the economic wealth of the European Community (EU) (http://ec.europa.eu/environment/iczm/pdf/2000brochure\_en.pdf). Urban settlements, industrial and agricultural activities, fishing, commercial traffic and tourism reduce vital space and introduce high quantities of nutrients and pollutants along the 89,000 kilometers of the European coasts, increasing eutrophication and pollution. TWS are mainly affected by anthropogenic impacts because of their shallow bottoms and closed morphology that rarely allows suitable hydrological renewal. These environments, which host habitats and species of conservation interest, are often severely degraded and require special attention. For this reason, they are monitored and have become the subject of numerous studies to understand and try to reverse the causes of their degradation.

The north-western Adriatic Sea is a closed shallow basin where various rivers flow, draining the Po plain and forming large TWS. From North to South three main lagoon systems are present: the lagoons of Marano-Grado, the lagoon of Venice and the lagoons and ponds of the Po Delta. Among them, the lagoon of Venice is the largest and most studied TWS in the Mediterranean Sea. The first biological studies of the lagoon date back to the end of the eighteenth century (Olivi 1794, Naccari 1828, Zanardini 1841 and subsequent papers of the same author, Meneghini 1842 and subsequent papers, De Toni 1889 and subsequent papers, Sighel 1938, Schiffner and Vatova 1938). They mainly dealt with the presence of macroalgae (Sfriso and Curiel 2007, Sfriso et al. 2009), showing only taxonomic data but without mentioning either the quantitative distribution of the species or any environmental parameter. The first studies of the species distribution and the monitoring of environmental parameters throughout the Venetian lagoon were made by Giordani-Soika and Perin (1970, 1974a, b), Perin (1975) and Perin et al.(1983) and refer to the distribution of some benthic taxa (some bivalves and polychaetes) and some nutrients (ammonium, orthophosphate, total phosphorus and total nitrogen) in the water column and surface sediments of the lagoon in 1948 and 1968. These data are the reference parameters to study the changes which have been recorded in the lagoon since the middle of the twentieth century. Later, studies began to address all the aspects concerning the lagoon. Regarding the macrophytes, nutrients and the main physico-chemical parameters of water and sediments, dozens of works are available. Maps of the macroalgal distribution and density have been drawn since 1980 (Sfriso and Facca 2007) whereas maps of nutrients in the water column and environmental parameters in the surface sediments date back to 1983 (Battaglia et al. 1983, Cossu and De Fraja-Frangipane 1985, Sfriso et al. 1995, 2003) for the central lagoon and to 1985 for the whole lagoon (Cossu and De Fraja-Frangipane 1985, Sfriso et al. 1988, Sfriso et al. 2003, Facca et al. 2014).

The present work investigates the way nutrient concentrations, macroalgal biomass and the environmental parameters of water column and surface sediments have changed since the middle of the last century. The aim is to explore the large dataset and highlight the most relevant parameters necessary to monitor trophic changes, in order to contribute to the institutional monitoring and environmental agencies' achievement in obtaining significant results with reduced efforts and lower costs.

## Materials and methods

# Sampling sites

The lagoon of Venice (https://deims.org/f7d94927-17be-4d3d-9810-e3c9bc91829c) is a polyhedric shallow water body located in the northern Adriatic Sea which has a water surface of ca. 432 km² and a mean depth of ca. 1.2 m (Fig. 1). The lagoon is connected to the sea through three large (400–900 m) and deep (15–50 m) mouths which divide it into three hydrological basins separated by watersheds which shift according to tides and winds. The present study refers to the three morphological basins: northern, central and southern lagoon. Burano and Torcello tidal marshes mark the separation between the central and the northern basin and the deep Malamocco-Marghera artificial canal the separation between the central and the southern one. The central basin (ca. 132 km²) has been the most studied because it suffered the impact of industrial waste, urban sewage and other anthropogenic pressures such as commercial and touristic activities and the illegal fishing of Manila clam (*Tapes philippinarum* Adams & Reeve) by disruptive fishing gears (Sfriso et al. 2003).

# Physico-chemical parameters, nutrient concentration and macroalgal biomass

Information on data collected before 1980 is reported in the cited papers whereas the surveys carried out by our research team, sampling procedures and analytical methods are summarized in the following pages.

Data on nutrients, macroalgal biomass and environmental parameters of the water column have been collected in the whole central lagoon since 1987 (34 sites). Monitoring surveys were carried out in 1993 (34 sites), 1998 (52 sites), 2003 (65 sites), 2011 (45 sites) and 2014 (34 sites). Data of ammonium concentration were also reported for the whole lagoon in 2011 (118 sites).

Thirty-four sites in early summer 1987, 1993, 1998 and 2003, and 31 sites in 2011 were monitored by collecting the 5 cm surface sediment top layer for phosphorus, total nitrogen analyses and determination of the sediment density.

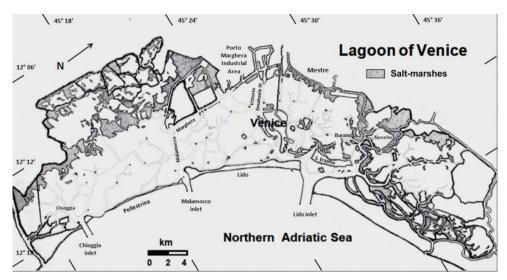


Figure 1. Map of the Venice Lagoon.

At each site, dissolved oxygen was measured with a portable instrument (Oxi 196 oximeter, Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany). Oxygen concentrations were reported as percentage of saturation (%DO) taking into account water temperature and salinity. Water transparency was measured with the Secchi disk. Transparency measurements were reported as a percentage of water column visibility because the waters are shallow (0.5–2.5 m) and the tidal excursion is relatively high (0.5–1 m). A value corresponding to 100% means that the bottom was visible, 50% means that the disk disappeared half way to the bottom. pH and Eh were measured with a portable pH-meter (pH 25) of the CRISON Instruments (Barcelona, Spain).

Six water column samples were collected with a home-made cylindrical sampler (length: 1.50 cm, diameter: 4 cm) which was repeatedly plunged into the water and poured to a tank. Sub-samples of 0.1–1.0 L were filtered through GF/F Whatman glass fiber filters (porosity: 0.7 mm). Filters and water samples were stored frozen at -18 °C for chlorophyll-a (Chl-a) analyses (Lorenzen 1967) and nutrient (reactive phosphorus (RP) and dissolved inorganic nitrogen (DIN) as the sum of ammonium, nitrite and nitrate) determination according to Strickland and Parsons (1984). Other water sub-samples were collected for salinity, which was determined in the laboratory by chlorine titration according to Oxner (1962).

At each site three sediment cores (first 5 cm top layer) were collected with a Plexiglas corer (i.d. 10 cm) and carefully mixed together. Sub-samples were retained to determine density and nutrient (total nitrogen and total, inorganic and organic phosphorus) concentrations. Density was obtained as g cm<sup>-3</sup> of wet and dry sediment according to Sfriso et al. (2003).

Total nitrogen (TN) concentrations were obtained by a Flash 2000 CHNS Analyser (Thermo Fisher Scientific spa), sediment freezing, lyophilization and pulverization. Inorganic ( $P_{inorg}$ ) and total phosphorus ( $P_{tot}$ ) were, respectively, determined before and

after combustion at 550 °C for two hrs, with dissolution in 1N HCl and spectrophotometric measurements according to Aspila et al. (1976). Organic phosphorus ( $P_{org}$ ) was obtained by difference. All the analyses were carried out in duplicate on two different days and values were retained when the difference was <5%. To compare the nutrient concentrations of the surface sediments of each site, values were normalized with reference to the amount of dry sediment per volume unit (dry density).

The macroalgal biomass was sampled between late May and July, which is the period when it is most abundant. At each site, biomass samples were collected with a 71×71 cm square frame or with a rake when the biomass was low (3–6 replicates) according to Sfriso et al. (1991, 2014a). Samples were weighted with a mechanical/electronic balance according to the biomass amount. The number of sampling sites ranged from 65 (1987), when macroalgae were very abundant and widespread, to 34 (2014).

# Statistical analyses

Data of different datasets of the whole central lagoon have been compared and means, standard deviations, maximum and minimum values have been determined. The Shapiro-Wilk test differentiated non-normal data, and then Friedman one-way ANOVA values (p <0.05) and Spearman's non-parametric coefficients have also been calculated. The basic statistics and correlation analyses of the data collected in the 5 periods were carried out both separately (1987, 1993, 1998, 2003, 2011) and by considering the whole data set (1987–2011). The total information has been summarized in a table reporting the number of significant (p<0.05) correlations per single parameter and period. The principal component analysis (PCA) has been applied to log-transformed data to visualize the variance and the association between parameters of both single and total periods, and to explore the scores with a loading >0.7, which is the generalized standard used for this statistical analysis. Total data are visualized in a bi-plot, whereas the loadings >0.7 of the single variables of each period are reported in Table 5 showing the parameters with the highest variance in decreasing order.

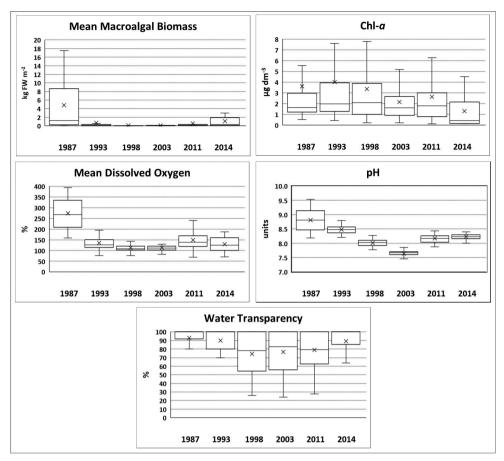
The similarities and differences between the stations during the 5 sampling periods have been investigated by analyzing the same data in a transposed matrix and bi-plotting the results. The stations are grouped according to their ecological characteristics and comparisons between the separated or overlapped periods highlight their differences.

Data were processed by Statistica software, Release 10 (StatSoft Inc. Tulsa, USA) provided by an academic license.

## Results

# Macroalgal biomass and environmental parameters

The first detailed distribution of macroalgae over the whole lagoon took place in 1980 and was replicated in 2003 (Sfriso and Facca 2007). During this period the biomass



**Figure 2.** Trends of the mean, standard deviation, maximum, minimum and median values of the macroalgal biomass, Chl-*a*, dissolved oxygen, pH and water transparency in the central lagoon of Venice.

decreased by ca. 90% in the whole lagoon and by over 96% in the central lagoon. However, additional information is available for the central lagoon (ca. 132 km²) because the biomass was mapped in 1987, 1993, 1998, 2003, 2011 and 2014, together with some physico-chemical parameters and the concentrations of nutrients in the water column and surface sediments. The highest mean biomass, mainly represented by *Ulva rigida* C. Agardh and *Ulva laetevirens* Areschoug, was recorded in late spring in 1987. It showed a mean value of 4.78 kg FW m⁻² (65 sites, Fig. 2) and a density that in many sites ranged between 10 and 20 kg FW m⁻², reaching even 25 kg FW m⁻² in one site. The mean biomass markedly decreased to 0.69 kg FW m⁻² in 1993 and to 0.11 kg FW m⁻² in 1998 (one-way ANOVA: p<0.001). In the following years, the biomass slowly increased, reaching a mean value of 1.05 kg FW m⁻² in 2014 (one-way ANOVA: p<0.001). In the same periods, phytoplankton, recorded as Chl-*a*, was expected to increase. On the contrary, the mean concentration of Chl-*a* showed similar values in 1987 (3.61±5.62 μg dm⁻³), 1993 (4.01±5.43 μg dm⁻³) and 1998 (3.36±4.45 μg dm⁻³) and decreased significantly (1.32±1.58 μg dm⁻³) in 2014 (one-way ANOVA: p<0.05) (Fig. 2).

The environmental parameters mainly affected by the presence of macroalgae (i.e. the concentration of dissolved oxygen, pH and water transparency) showed a strongly related trend to the biomass variation (Fig. 2). In 1987, due to the high presence of macroalgal biomass, the mean %DO saturation was 274%, ranging between 159 and 394%. In 1993, when the biomass decreased significantly, the mean %DO halved to 135% (one-way ANOVA: p<0.001) and in the following periods fluctuated between 113 and 150% (one-way ANOVA: p<0.001). In 1987, the lowest %DO was higher than the mean values of the following periods. Moreover, since 1993 the lowest values have always been undersaturated. The pH values showed a similar decreasing trend (Fig. 2) with the highest mean value in 1987 (8.81) and the lowest one in 2003 (7.64) (one-way ANOVA: p<0.001). In contrast, the presence of high biomass, especially represented by laminar Ulvaceae that hamper sediment resuspension, enhanced water transparency which decreased significantly in 1998 and 2003 when the effort of clam harvesting was at its highest.

#### Nutrient concentrations

Changes of nutrient concentrations in the surface sediments of the Venice lagoon are reported in Table 1. The first data of total phosphorus ( $P_{tot}$ ) and total nitrogen ( $N_{tot}$ ) in the surface sediments date back to 1948–49 (Perin 1975, Perin et al. 1983), before the  $2^{nd}$  post-war industrial development. The highest changes were recorded for  $P_{tot}$  concentrations. During the period 1948–49,  $P_{tot}$  presented very low values:  $24\pm16~\mu g~g^{-1}$  (mean $\pm SD$ ). Twenty years later, in the period 1968–73, this value increased remarkably ( $164\pm79~\mu g~g^{-1}$ ) and even more in 1983 ( $454\pm126~\mu g~g^{-1}$ ), when it reached the highest mean concentration, i.e. 18.9 times the concentration measured in 1948–1949. In the following years,  $P_{tot}$  mean concentration decreased slightly, showing average values between 339 and 375  $\mu g~g^{-1}$  (Cossu and De Fraja-Frangipane 1985; Sfriso et al. 2003, Facca et al. 2014). The maximum value was recorded by Cossu and De Fraja-Frangipane (1985): 1102  $\mu g~g^{-1}$ . No record for the organic and inorganic fractions is available.

Total Nitrogen ( $N_{tor}$ ) exhibited a mean value of  $1.00\pm0.86$  mg  $g^{-1}$  in 1948–49, increasing up to  $1.86\pm2.20$  mg  $g^{-1}$  in the period 1968–73, followed by a progressive decrease to  $0.69~\mu g~g^{-1}$  in 2011. The maximum value was recorded in the period 1968–73 by Perin (1975) with  $3.56~m g~g^{-1}$ .

Some more detailed information is available for the central basin of the Venice lagoon where the same operators monitored the surface sediment top layer (5 cm) of 31–34 stations in successive late spring-early summer periods, characterized by different scenarios (1987: presence of high algal biomass; 1993: sharp reduction of macroalgal biomass; 1998: intense Manila clam (*Tapes philippinarum* Adams and Reeve) harvesting; 2003: the highest Manila clam harvesting; 2011: sharp reduction of clam stocks (from ca. 40,000 tonnes in 2010 to ca. 2000 tonnes in 2012) and decrease of clam fishing activities).

During the period between 1987 and 2011 the mean concentrations of  $P_{tot}$  per volume unit (µg cm<sup>-3</sup> of sediment) did not change significantly, except for the maximum value of 720 µg cm<sup>-3</sup> in 1987, then progressively decreased to 473 µg cm<sup>-3</sup> (-34%) in

			1948-	2011						
		Sediment thickness		Phos	phorus			Nitr	ogen	
		cm	Mean		SD	Max	Mean		SD	Max
					μg g <sup>-1</sup>				mg g <sup>-1</sup>	
Perin 1974	1948-49	30	24	<u>+</u>	16	50	1.00	±	0.86	1.96
Perin 1974	1968-73	30	164	$\pm$	79	250	1.86	$\pm$	2.20	3.56
Perin et al. 1983	1983	20	454	$\pm$	126	682	1.33	$\pm$	0.59	2.74
Cossu and De Fraja-	1987-88	20	339	$\pm$	215	1102	1.33	$\pm$	0.89	4.80
Frangipane 1995										
Sfriso et al. 2003	1987	5	386	$\pm$	96	720	1.21	$\pm$	0.60	3.00
Sfriso et al. 2003	1993	5	361	$\pm$	80	682	1.14	$\pm$	0.48	2.62
Sfriso et al. 2003	1998	5	375	$\pm$	65	541	0.93	$\pm$	0.48	1.37
Facca et al. 2009	2003	5	358	$\pm$	99	635	0.71	$\pm$	0.36	1.48
Facca et al. 2014	2011	5	367	$\pm$	114	896	0.69	$\pm$	0.75	2.89

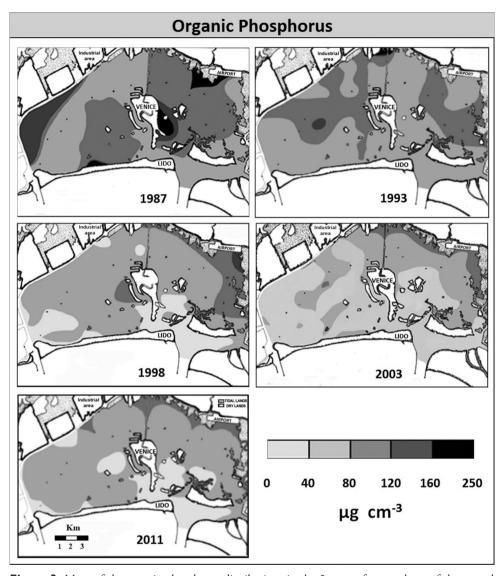
**Table 1.** Nitrogen and phosphorus changes in surface sediment top layer (5 cm) in the whole lagoon.

Table 2. Changes of Total Phosphorus, Organic Phosphorus and Total Nitrogen in the central lagoon.

			T	otal Phosphor	rus	
	1987	1993	1998	2003	2011	changes
			μg/cm <sup>3</sup>			
site Nº	34	34	34	34	31	%
Mean	386	361	375	358	383	≈
SD	96	80	65	99	50	
Min	227	184	257	201	281	
Max	720	682	541	635	473	-34
			Or	ganic Phosph	orus	
	1987	1993	1998	2003	2011	changes
			μg/cm³			
site Nº	34	34	34	34	31	%
Mean	104	67	59	53	62	-40
SD	42	28	31	53	24	
Min	49	27	16	2	13	
Max	246	210	167	150	113	-54
				Total Nitroge	n	
	1987	1993	1998	2003	2011	changes
			mg/cm <sup>3</sup>			
site N°	34	34	34	34	31	%
Mean	1.21	1.14	0.93	0.71	0.35	-71
SD	0.60	0.48	0.48	0.36	0.48	
Min	0.22	0.33	0.10	0.09	0.04	
Max	3.00	2.62	1.37	1.48	0.48	-84

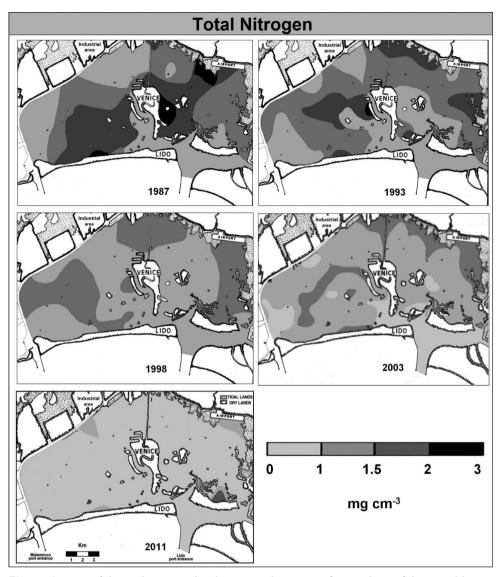
2011 (Table 2). In contrast, the mean value of the organic fraction ( $P_{org}$ ) lowered by ca. 40% (from 104 to 62 µg cm<sup>-3</sup>, one-way ANOVA: p<0.001). On the whole, the ratio  $P_{org}/P_{tot}$  decreased from 26.9% to 16.2% and in 2011 the maximum  $P_{org}$  concentration was more than halved (from 246 to 113 µg cm<sup>-1</sup>).

In the same period (1987–2011), the mean  $N_{tot}$  concentration decreased from 1.21 to 0.35 mg cm<sup>-3</sup> (ca. -71.1%, one-way ANOVA: p<0.001) whereas the peak concentration lowered by 32%, from 3.00 to 2.05 mg cm<sup>-3</sup>, although the lowest value was recorded in 1998 with 1.37 mg cm<sup>-3</sup>.



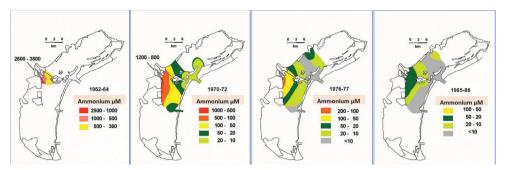
**Figure 3.** Maps of the organic phosphorus distributions in the 5 cm surface top layer of the central lagoon basin in 1987, 1993, 1998, 2003 and 2011.

Figs 3, 4 highlight the variation of  $P_{org}$  and  $N_{tot}$  loads in surface sediments of the central lagoon. In the first survey (1987) organic phosphorus showed high concentrations in 4 areas, one of which was affected by the industrial waste of Porto Marghera and the others by the urban discharges of Venice, Lido, Mestre and its hinterland (Fig. 3). In the following years,  $P_{org}$  concentrations decreased progressively and in 2011 the highest values were recorded along the mainland coast. Total nitrogen showed a temporal decrease even greater. Except for a station located at the north of the island of S. Erasmo, it generally decreased below 1 mg cm<sup>-3</sup> (Fig. 4).

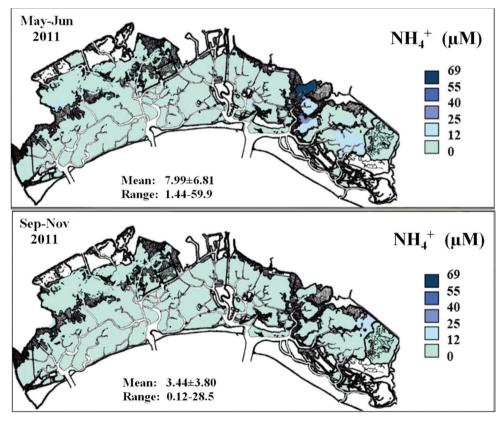


**Figure 4.** Maps of the total nitrogen distributions in the 5 cm surface top layer of the central lagoon basin in 1987, 1993, 1998, 2003 and 2011.

A significant decrease was also observed for the concentrations of nutrients in the water column Fig. 5). The first records date back to the period 1962–64 and deal with the concentration of ammonium in the canals of Porto Marghera industrial area and the lagoon surface in the vicinity. These values were very high (1000–2500  $\mu$ M), reaching even 3800  $\mu$ M in the industrial canals (Fig. 5). Ammonium concentration decreased to 500–1000  $\mu$ M in the period 1970–72, lowering to 50–100  $\mu$ M in 1985–8 (Fig. 5). In 2011 (Fig. 6) and 2014 the highest value recorded in that area was <10  $\mu$ M.

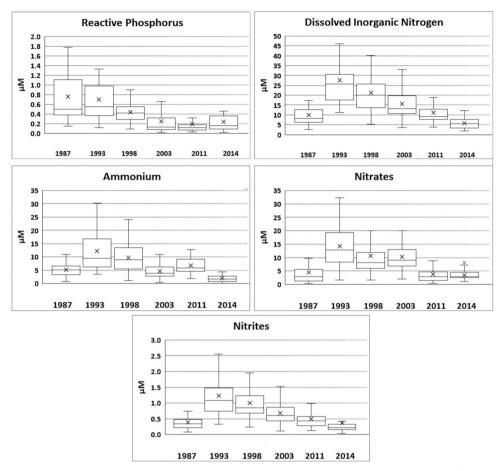


**Figure 5.** Distribution of ammonium in the water column in the central lagoon from 1962 to 1986 (from Cossu and De Fraja-Frangipane 1985).



**Figure 6.** Map of ammonium distribution obtained for the whole lagoon by sampling 118 sites in spring and in autumn 2011.

By considering the whole central lagoon, information on the nutrient concentrations in the water column has been available since 1987. The mean concentration of reactive phosphorus (RP) decreased from 0.76  $\mu$ M in 1987 (34 sites) to 0.19  $\mu$ M in 2011 (45 sites), slowly increasing to 0.24  $\mu$ M in 2014 (34 sites, Fig. 7) (one-way



**Figure 7.** Trends of the mean, standard deviation, maximum, minimum and median values of the reactive phosphorus, dissolved inorganic nitrogen, ammonium, nitrate and nitrite concentrations in the central lagoon of Venice.

ANOVA: p<0.001). Conversely, the concentration of the dissolved inorganic nitrogen (DIN) was relatively low in 1987 (10.1  $\mu M$ ), in the presence of a very high macroalgal biomass, but it remarkably increased up to 27.6  $\mu M$  in 1993 (32 sites) when macroalgae were negligible. Afterwards, DIN concentration progressively decreased to 5.9  $\mu M$  in 2014 (one-way ANOVA: p<0.001). The mean concentrations of ammonium and nitrates showed the same pattern, increasing from 1987 (5.15 and 4.54  $\mu M$ , respectively) to 1993 (12.2 and 14.2  $\mu M$ , respectively) and decreasing in the following years to 2.01  $\mu M$  (ammonium) and 3.51  $\mu M$  (nitrates) (one-way ANOVA: p<0.001). Similarly, nitrites showed a mean concentration of 0.38  $\mu M$  in 1987, increasing to 1.23  $\mu M$  in 1993 and decreasing again to 0.37  $\mu M$  in 2014 (one-way ANOVA: p<0.001).

# Statistical analyses

The Spearman non-parametric correlation matrices of data collected in the central lagoon were determined by considering both the whole period: 1987-2011 and the years 1987, 1993, 1998, 2003, 2011, separately. On the whole, the highest number of significant (p<0.05) direct or inverse correlations was shown by RP (13 out of 18), salinity, Chl-a and nitrite (12 out of 18) and pH (11 out of 18) Tables 3, 4). Results depended on the different scenarios. In 1987, the highest number of correlations was found with temperature; in succession, other parameters were nitrite, macroalgal biomass and some parameters associated with the presence of biomass such as %DO,  $P_{tot}$  and  $P_{org}$  accumulated in the surface sediments and DIN in the water column. In 1993, macroalgal biomass started to decrease significantly and in 1998, when the biomass was negligible and clam fishing activities were affecting a wide part of the central lagoon, the parameters with a high number of significant correlations were salinity (12), water transparency (8) and  $P_{inorg}$ ,  $P_{tot}$  (10 and 8, respectively). In 2003, when clam fishing showed the highest efforts, water transparency (13) dropped down and high amounts of ammonium (15) and P<sub>org</sub> (12) were released by the surface sediments. RP continued to decrease (12) in the water column whereas pH (13) showed the lowest values (Fig. 7). In 2011, the scenario changed and the number of correlations decreased (Table 4).

The principal component analysis highlighted the parameters with the highest variance (Table 5, Fig. 8). Their spatial distribution and association is displayed by bi-plotting the values of the first two components. The analysis of the whole dataset (1987–2011) shows the parameters with a loading >0.7 (Table 5). Among them, RP and nitrite (NO<sub>2</sub>-) played a key role in the changes of the environment as well as %DO (O<sub>2</sub>) and P<sub>org</sub> (Fig. 8). Moreover, the macroalgal biomass was associated with %DO, pH, water transparency, salinity and sediment density, and opposed to nutrient concentrations, Chl-*a* and water temperature. The analysis of the parameters of each monitoring period is reported in Table 5. On the whole, salinity displayed a loading >0.7 each single year but the value changes when the whole period is considered. Similarly, nitrate and water transparency showed significant loadings (4 out of 5) in the single periods, but not when they referred to the whole period. Nitrite, pH, P<sub>org</sub> loadings were significant in three periods and only P<sub>org</sub> did not display any significant value. These trends are also highlighted when the significant values of each parameter are summed.

The bi-plot of the first two PCA components of the transposed matrix highlights the similarities/dissimilarities between the stations of the different sampling periods (Fig. 9). All the stations sampled in 1987 and 1993 are opposite to the ones sampled in 2011. In contrast, results of sampling carried out in 1998 and 2003 partly overlap the ones occurred in other periods highlighting that the environmental conditions in 1998 and 2003 were intermediate.

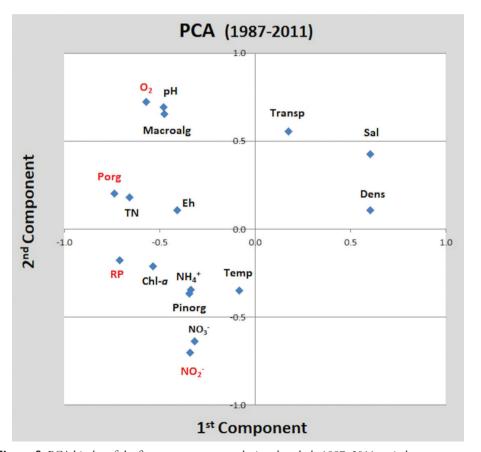
Table 3. Spearman non-parametric coefficients for the total period 1987–2011 in the central lagoon.

Parameters	Parameters Temperature	Hd	Eh	0	Salinity	Salinity Transparency		Chl-a Macroalgae	RP	Ammonium Nitrite	Nitrite	Nitrate	DIN	Density	P tot	Nitrate DIN Density P tot P inorg	P org	IN
Temperature	1.00																	
μd		1.00																
Eh		0.26	1.00															
0,		0.63	0.41	1.00														
Salinity	-0.15	0.12	-0.19	-0.07	1.00													
Transparency		0.37	0.08	0.33	0.40	1.00												
Chl-a		0.07	0.39	0.23	-0.56	-0.27	1.00											
Macroalgae		0.34	0.12	0.35	0.15	0.29	-0.14	1.00										
RP		0.36	0.37	0.22	-0.45	-0.06	0.43	-0.11	1.00									
Ammonium		0.16	-0.06	-0.11	-0.15	-0.03	0.15	-0.25	0.32	1.00								
Nitrite		-0.23	0.05	-0.30	-0.42	-0.23	0.31	-0.41	0.40	0.45	1.00							
Nitrate		-0.25	0.24	-0.15	-0.39	-0.11	0.35	-0.27	0.45	0.22	0.75	1.00						
DIN		-0.12	0.15	-0.19	-0.37	-0.11	0.35	-0.34	0.47	69.0	0.80	0.84	1.00					
Density		-0.06	-0.12	-0.07	0.44	0.27	-0.27	0.04	-0.25	-0.07	-0.18	-0.04	-0.07	1.00				
P tot		0.09	0.03	0.03	-0.42	-0.34	0.21	0.00	0.22	0.09	90.0	-0.07	-0.04	-0.44	1.00			
P inorg		-0.11	-0.08	-0.15	-0.29	-0.40	0.08	-0.18	0.11	0.09	0.14	-0.04	-0.01	-0.28	0.87	1.00		
P org		0.32	0.14	0.27	-0.41	-0.15	0.32	0.23	0.32	0.13	0.01	-0.05	0.00	-0.56	99.0	0.28	1.00	
ZI		0.27	0.37	0.34	-0.27	0.05	0.41	90.0	0.32	0.11	0.17	90.0	0.11	-0.44	0.32	0.12	0.52	1.00

In bold p<0.05 per r≥0.16

<b>Table 4.</b> Number of correlations among pa	parameters.
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	1997-2011	1987	1993	1998	2003	2011
RP	13	6	8	1	12	5
Salinity	12	7	9	12	9	6
Nitrite	12	8	4	6	11	6
Chl-a	12	2	8	6	10	1
pН	11	4	7	5	13	7
Porg (Sed)	10	7	9	7	12	4
$O_2$	10	7	9	4	11	2
Nitrate	10	4	5	4	9	5
TN	10	2	5	7	6	2
DIN	9	7	6	7	9	7
Transparency	9	3	6	8	13	4
Density (Sed)	9	4	8	7	7	4
Macroalgae	9	7	1	0	6	2
Ptot (Sed)	8	7	8	8	9	2
Eh	7	1	2	5	0	1
Ammonium	6	3	2	8	15	1
Pinorg (Sed)	6	5	5	10	8	1
Temperature	3	9	0	5	0	0

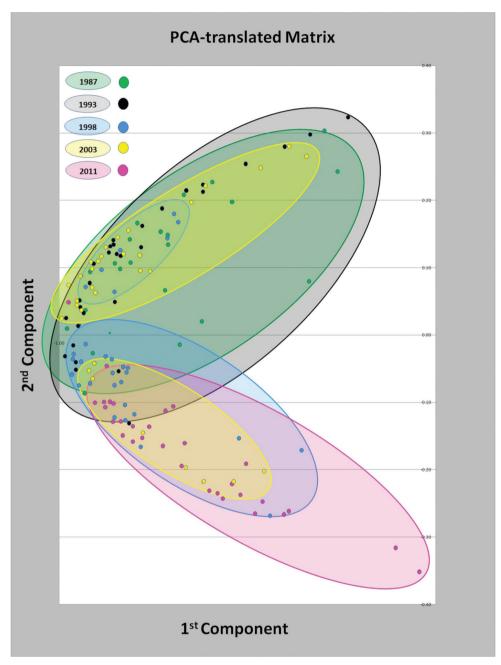


**Figure 8.** PCA bi-plot of the first two components during the whole 1987–2011 period.

 Table 5. PCA loadings of the whole period and the single years.

Periods Salinity Nitrate Trans	Salinity	Nitrate	Transparency	Nitrite	Hd	P org	RP	temperature	Chl-a	IN	0,	Ammonium	Eh	Density	ensity Macroalgae	P inorg
1987–2011	0.61	0.64	0.35	0.70	69.0	0.74	0.71	0.35	0.54	99.0	0.72	0.34	0.41	09.0	0.65	0.37
1987	0.75	0.38	0.63	0.62	92.0	0.73	0.65	0.85	0.46	0.31	0.74	0.46	0.43	0.59	0.62	0.54
1993	0.90	0.71	0.74	0.78	0.64	0.61	0.74	0.22	0.85	0.57	0.75		0.45	0.55	0.22	09.0
1998	0.70	0.83	0.83	0.63	29.0	92.0	0.34	0.77	0.74	69.0	0.55	0.64	0.84	0.81	0.55	09.0
2003	0.77	92.0	0.77	0.88	0.87	0.84	0.90	0.35	99.0	0.75	0.65		0.20	0.63	0.30	0.62
2011	92.0	0.78	0.72	98.0	0.73	0.62	09.0	0.20	0.51	0.74	0.56		0.56	0.65	92.0	0.55
Total single	3.88	3.46	3.69	3.77	3.67	3.56	3.23	2.39	3.22	3.06	3.25		2.48	3.23	2.45	2.91
years																

in bold = loading >70



**Figure 9.** Transposed PCA bi-plot of the first two components during the whole 1987–2011 period. The separation or overlapping of the different years are highlighted by different colours.

## **Discussion**

The lagoon of Venice is one of the most studied transitional environments of the Mediterranean Sea and the high number of available datasets enables us to understand environmental changes over the years and foresee its evolution. The trophic parameters and primary producers linked to their change have been studied assiduously since the early '80s, although for some of them, data have been available since the late '40s. Actually, since the 2<sup>nd</sup> post-war industrial development frequent changes of the environmental scenarios have witnessed the effect of different anthropogenic activities. Between the '60s and '80s, a significant increase of eutrophication was recorded (Wolf 1986, Dunnette and O'Brien 1992, Schramm and Nienhuis 1996). In Italy, it affected firstly freshwater environments, especially shallow lakes and ponds (Chiaudani et al. 1980), then transitional (Cossu et al. 1983, 1984, Facco et al. 1986, Sfriso et al. 1988) and coastal (Chiaudani et al. 1980, 1983) environments. The Venice lagoon, because of its historical and economic role, was the object of a very high number of environmental studies. In fact, hydrological interventions like the digging of wide and deep commercial canals (i.e. Vittorio Emanuele III, Malamocco-Marghera, namely "Canale dei Petroli"), the development of the industrial area of Porto Marghera, the increase of agricultural monoculture and population living in urban centers dramatically increased the flow of nutrients and pollutants into the lagoon (Marcomini et al. 1993). The first effect was a sharp increase in their concentration both in the water column and surface sediments which led to a change of primary producers and the spreading of nuisance macroalgae (Sfriso et al. 1988, Morand and Briand 1996, Ménesguen 2018). Since the early '90s, some synergic factors, mainly driven by climatic changes, have affected macroalgal growth (Sfriso and Marcomini 1996) that rapidly declined. In the meantime, the clam Tapes philippinarum, which had been introduced in the lagoon for economic purposes (Cesari and Pellizzato 1985), spread everywhere and fishing activities strongly affected the sediment texture by changing its grain-size and benthic communities (Pranovi et al. 2004, Sfriso et al. 2005). The main effect was the resuspension of high amounts of sediments, nutrients and pollutants (Sfriso et al. 2003, 2005). This scenario lasted until clam stocks were depleted and the lagoon began a progressive recovery of its ecological conditions which is still underway. In addition, in the past twenty years, many decrees and directives have contributed to reduce the trophic status of the lagoon (Ronchi-Costa Decree April 23th 1998, Water Framework Directive (2000/60/EC, etc.) which showed a remarkable resilience (Regione Veneto et al. 2012, 2015, Facca et al. 2014). Nowadays, the anthropogenic pressures that in the past used to affect the lagoon have decreased, especially nutrient enrichments and clam fishing, and the trophic conditions of the basin are regulated by the low concentrations of phosphorus. This element was banned from the detergent formulations (4-5% of total weight) in 1989 (Solidoro et al. 2010) and is presently the main factor limiting the primary production in the lagoon. Therefore, the Venice lagoon differs from many transitional and coastal areas that are affected by increasing or steady high trophic conditions, such as the lagoons and ponds of the Po delta and the Valli di Comacchio (Munari and Mistri 2012, Sfriso et al. 2014b), the costs of Chine (Hu et al. 2010, Ye et al. 2011), the coasts of Brittany (Diaz et al. 2013, Perrot et al. 2014, Ménesguen 2018) the gulf of Mexico (Rabalais et al. 2009) and many others, and showed a return to good/high environmental conditions. The improvement of the environment is also highlighted by the increase of seagrass cover and the spreading of sensitive macroalgal taxa which in the Venice lagoon occurred both naturally and thanks to special projects. Recently, the project SeResto (Seagrass Restoration) developed a new strategic approach to meet HD & WFD objectives" (Bonometto et al. 2018, Sfriso et al. 2018) by transplanting aquatic angiosperms. The project funded by the European Community in the framework of LIFE12 NAT/IT/000331"Habitat 1150\* (Coastal lagoon) recovery by SEagrass RESTOration" contributed to colonizing ca. 10 km<sup>2</sup> of the lagoon at different levels of coverage, ca. 4 km<sup>2</sup> of total plant cover, thus recovering fish and benthic communities. As the success of the project was due to the general recovery of the lagoon it is believable that in the absence of additional anthropogenic pressures, the environment should continue to improve its ecological conditions as evidenced by the monitoring of biological elements in accordance with WFD requirements (Regione Veneto et al. 2015).

## **Conclusions**

This paper was prepared in the framework of the LTER-Italy network in order to analyze a part of the great amount of data collected in the Venice lagoon. The long-term analysis of the trophic status since the middle of the 20th century made it possible to highlight both the evolution of this environment and the parameters related to the observed changes. The lagoon eutrophication increased markedly from the 2<sup>nd</sup> postwar period until the end of the '80s, when high nutrient amounts were released into the environment (dissolved in the water column and accumulated in the surface sediments), triggering macroalgal blooms and favoring hyper-dystrophic conditions. The parameters which are related to the primary production (%DO, pH, water transparency, RP, P<sub>ore</sub>) showed the highest changes. In the following years, different environmental scenarios have occurred but the latest data show that the lagoon environment is improving and, without other additional anthropogenic pressures, it should keep a positive trend for at least 10 years or longer. Results highlight that the trophic status of a transitional environment, from the physico-chemical point of view, can be easily detected by measuring some driver parameters such as RP and nitrites. They are the most sensitive nutrients to the environmental changes, easy to analyze and low-cost. The analysis of these two parameters together with the measurement of pH and the oxygen concentration can support macrophyte assemblages for the assessment of the ecological status according to the WFD (2000/60/EC) and provide an exhaustive method for the determination of the trophic status of a transitional water system.

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