Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Marine Pollution Bulletin 54 (2007) 1607–1618
MARINE POLLUTION BULLETIN
www.elsevier.com/locate/marpolbul

Application of biotic indices and relationship with structural and functional features of macrobenthic community in the lagoon of Venice: an example over a long time series of data

Fabio Pranovi *, Filippo Da Ponte, Patrizia Torricelli

Dipartimento di Scienze Ambientali, Università Ca' Foscari, Castello 2737/B, 30170 Venice, Italy

Abstract

In the context of the application of WFD, a scientific debate is growing about the applicability of biotic indices in coastal and transitional waters. In the present work, the question about the discriminating power of different biotic indices and the relationships with the structure and functioning of the macrobenthic community in a transitional environment is discussed. A time series of samples collected during the last 70 years in the lagoon of Venice, reflecting different environmental conditions (a sort of 'pristine state' in 1935, the distrophic crisis in 1988 and subsequent modifications in 1990, the invasion by an alien species and the developing of high impacting fishery in 1999) has been used. The comparison of results obtained by applying different biotic indices, such as AMBI, Bentix and BOPA, shows differences in the discriminating power of indices and a general overestimation of environmental conditions. Discrepancies between environmental status as indicated by biotic indices and the structure and functioning of the benthic community have been highlighted.

Keywords: Biotic indices; Diversity; Exergy; Ecosystem functioning; Lagoon of Venice

1. Introduction

The implementation of the European Water Framework Directive (WFD, 2000/06/EC; EC, 2000) has produced, on one side the developing of a series of common concepts, terminologies and tools, and on the other a sort of race to the development of 'new indices' (Dauvin, 2007).

In this context there is a scientific debate about the applicability of biotic indices, both 'old' and 'new', to determine the quality of European coastal and transitional waters, according to Ecological Quality Status (EcoQ) (Borja et al., 2000, 2003, 2004a,b; Borja and Heinrich, 2005; Simboura and Zenetos, 2002; Simboura, 2004; Simboura et al., 2005; Dauvin et al., 2007). Indeed, a biotic index is unlikely to be universally applicable, because all organisms are not equally sensitive to all types of anthropogenic disturbances and thus are likely to respond differently to different types of perturbations. Moreover, many of these indices are still dependent on the Pearson–Rosenberg model for organic enrichment; hence they must be validated for other stressors, such as physical disturbance and chemical pollution (Quintino et al., 2006). All this generates the so-called 'paradox of estuarine quality' (in Dauvin, 2007). Transitional estuarine waters are naturally organic rich environments where stress-tolerant species are typical, so transitional environments would therefore likely be, by definition, characterised by low scores and so low EcoQ values.

At present, in the context of environmental management/policy, there is an increase in the need for a more services-oriented scientific work, to assess effects of community structure changes (such as biodiversity loss) on services, but this implies the improving of the knowledge in the process-oriented ecology, bridging the gap between community ecology and ecosystem ecology, as suggested by Raffaelli (2006). It is necessary to improve the knowledge about the cause–effect relationships between changes in the structure of biological communities and ecological

* Corresponding author.
E-mail address: fpranovi@unive.it (F. Pranovi).

0025-326X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marpolbul.2007.06.010

processes, taking into account that all this operates at large spatial scales and generally involves many different trophic levels simultaneously. To face what is essentially an ecosystem-level question, an ecosystem-level approach is required (Raffaelli, 2006).

In this context, it is interesting to assess the applicability, in terms of discriminating capacity, of different biotic indices in particular for environments such as the lagoons, comparing obtained results with independent indications about the structure and functioning of the benthic compartment (see also JRC-EEWAI, 2007).

The aim of this paper is to compare the results of different indices, recently proposed for evaluation of the state of marine coastal environments, by using a time-series of macrobenthos samples collected in the Venice lagoon during the last 70 years. Results will be discussed in the light of the temporal pattern of main ecological driving forces and in the comparison between structure and functioning of the soft-bottom macrobenthic community of the lagoon.

2. Materials and methods

During the last century, the lagoon of Venice, one of the widest Mediterranean lagoon environments, has been subjected to intense anthropogenic pressures which have deeply modified it.

Three main events can be recognized:

- the modification of hydrodynamic conditions, with profound effects on habitat morphology in the 1960s (Ravera, 2000);
- the increase of nutrient load in the 1960s and 1970s (Cossu and De Fraja Frangipane, 1985), with eutrophication and subsequent macroalgae blooms and anoxia events recorded in the 1980s (Sfriso et al., 2003);
- the invasion by an alien species, Manila clam, at the end of the 1980s and the subsequent development of the mechanical clam harvesting in the 1990s (Cesari and Pellizzato, 1985; Provincia di Venezia, 2000; Pranovi et al., 2004).

2.1. Macrobenthic community data

The dataset (303 taxa, belonging to 8 different Phyla, and more than 450 samples) is composed from data collected by different surveys carried out in the lagoon during the last 70 years (Table 1) (for details about the database please refer to Pranovi et al., submitted for publication).

The time series is characterized by a heterogeneous distribution of samples through the time, with an important gap from 1935 to 1988, and by different survey's extension. Three surveys (1935, 1990 and 1999) covered the entire lagoon, whereas the other ones included one or two basins (Table 1, for the location of the basins see Fig. 1).

All collected species were checked for nomenclature, and data were standardized to m^2.

Table 1
Time series available for each basin of the lagoon

	1935	1988	1990	1995	1997	1999	2001	2004
Northern	x	x	x			x		x
Central	x	x	x	x		x	x	
Southern	x		x	x	x	x		

2.2. Marine biotic indices

In general terms, the theoretical basis of marine biotic indices is the community succession in a gradient of organic enrichment as proposed by Pearson and Rosenberg (1978), based on the concept that biological communities respond to environmental stress by means of different adaptive strategies. In the present study three different indices (AMBI, Bentix and BOPA) were tested.

AMBI (AZTI's Marine Biotic Index), as defined by Borja et al. (2000, 2003), is a biotic index which provides a classification of a site, representing benthic community health (sensu Grall and Glémarec, 1997). It is based on the distribution of the abundance of each species, into one of five ecological groups (EG), according to their sensitivity to environmental stress (mainly organic pollution). The index ranges between 0 (the best condition) and 7 (the worst one). Since, as reported also by Magni et al. (2005), better results in the assessment of EcoQ is achieved by applying a combination of different indices, the AMBI index was recently developed in a new version, called m-AMBI, to include in the assessment the number of species and the Shannon index (Muxika et al., 2007). For the index calculation the software available on http://www.azti.es was used.

Bentix is a biotic index proposed by Simboura and Zenetos (2002), based on the same idea as AMBI, but benthic species are grouped into two wider EG (the sensitive and the tolerant) (Simboura et al., 2005). The index can produce a series of continuous values from 2 to 6, being 0 when the sediment is azoic (all groups zero). The Bentix methodology and an extended list of species scores can be found on http://www.hcmr.gr/english_site/services/env_aspects/bentix.html.

BOPA (benthic opportunistic polychaetes amphipods) is an index, substantially based on the same ecological bases of previous ones, which uses the ratio between the frequency of opportunistic (tolerant) polychaetes and the frequency of amphipods to classify the state of a community (Gomez Gesteira and Dauvin, 2000; Dauvin and Ruellet, 2007). This index ranges between 0 (best condition, in the case of absence of opportunist polychaetes) and log2 (in the case of absence of amphipods).

In the context of the implementation of the European Water Framework Directive (WFD, 2000/06/EC), a growing scientific effort has been dedicated to the definition of the Ecological Quality Status (EcoQ) and its categorization (Borja et al., 2000, 2003, 2004a,b; Borja and Heinrich, 2005; Simboura and Zenetos, 2002; Simboura, 2004; Simboura et al., 2005; Dauvin et al., 2007). In Table 2, the

Fig. 1. Location of the three basins of the Venice lagoon.

ranges proposed for the biotic indices, used in the present study, to determine different classes of quality of ecological states are reported. Regarding the Bentix index, it should be noted that the scale reported applies for naturally stressed biotopes and was originally adopted for muddy environments, which are considered as naturally hosting many tolerant species as in the case of coastal lagoons (see Simboura and Zenetos, 2002; UNEP/MAP, 2005).

2.3. Community structure and functioning indices

To assess the community diversity in different times, besides a common diversity index, such as the Shannon index, the Average taxonomic distinctness ($\Delta+$), which represents the average path length between every pair of individuals in a sample (Clarke and Warwick, 2001) has been also computed.

To analyze the functioning of the benthic community, different approaches have been adopted.

An opportunity to analyse the functioning of a community is given by the way in which the energy is divided within the different components. In this context, exergy, which provide a thermodynamic metric that tracked the distance of the ecosystem from the thermodynamic equilibrium, could represent a useful measure (Marques and Jørgensen, 2002; Raffaelli, 2006). According to Odum (1969), during their development, self-organizing systems tend to increase biomass, structure, complexity and information, by transforming the free energy, therefore increasing their exergy.

Table 2
Classification of EcoQS according to ranges of AMBI, BENTIX, BOPA, H′, and m_AMBI; the Water Framework Directive status is also reported, according to references:AMBI (Muxika et al., 2005), BENTIX (UNEP/MAP, 2005), BOPA (Dauvin and Ruellet, 2007), H′ (UNEP/MAP, 2005), m-AMBI (Muxika et al., 2007)

Pollution classification	AMBI	BENTIX	BOPA	H'	m-AMBI	WFD status
Unpolluted/normal	≤ 1.2	4.0 – 6.0	≤ 0.046	>4.6	>0.80	High
Slightly polluted	1.3 - 3.3	3.0 – 3.9	0.045 - 0.140	4.1 - 4.6	0.60 – 0.80	Good
Moderately polluted	3.4 - 4.3	2.5 - 2.9	0.139 - 0.194	3.1 - 4.0	0.40 – 0.59	Moderate
Heavily polluted	4.4 - 5.5	2.0 -2.4	0.193 - 0.268	1.6 – 3.0	0.20 – 0.39	Poor
Extremely polluted/Azoic	5.6 - 6.0	B < 2	0.267 - 0.301	≤ 1.5	<0.20	Bad

Exergy for biological systems can be estimated by means of the following equation (Jørgensen et al., 1995; Bendoricchio and Jørgensen, 1997):

$$\text{Ex} = RT \cdot \sum_{i=0}^{N} (C_i \cdot \beta_i)$$

where R is the gas constant and T is the absolute temperature, C_i is the biomass concentration of the species (i) in the system and β_i the weighting coefficients expressing the information carried by the (ith) species. The genetic information was suggested as representing the information content embedded in biomass and thus a way of estimating the complexity and organization of organisms. At present, the debate about what measure should be used to represent the information carried by organisms is still open (Jørgensen et al., 1995; Marques et al., 1997; Fonseca et al., 2000; Debeljak, 2002). In this study, specific genome size (C-value) have been used, even if its use remains questionable since genome size includes the non-coding genes and the repeated DNA that are carried unused information (Bendoricchio and Jørgensen, 1997; Debeljak, 2002; Gregory, 2007).

To estimate C, the wet weight biomass data were transformed in ash free dry weight data according to transformation coefficients for main taxonomic groups proposed by Tumbiolo and Downing (1994). According to Marques et al. (2003), in the present study the relative exergy (exergy values divided for the total biomass) was applied.

In its original formulation, exergy is calculated as the distance from a reference state (Wall, 1977; Svirezhev, 2000) and the "primitive inorganic soup" was suggested as reference for biological systems (Jørgensen et al., 1995). For real ecosystems, however, a measure of local exergy (*sensu* Wall, 1977), defined in relation to a real reference state and/or to the surrounding environment appears to be more appropriate (Libralato et al., 2006). In the present study, exergy was evaluated by using the 1935 values as reference.

The trophic structure of the community was assessed by assigning each species to five trophic guilds (filter feeders, detritus feeders, herbivores, carnivores, omnivores/mixed feeders), using criteria such as the feeding apparatus morphology, the feeding mode and the nature and origin of the food (Fauchauld and Jumars, 1979; Desrosiers et al., 1986; Desrosiers et al., 2000; Todd, 2006).

In order to analyze the community in terms of life history, two different biological traits – adult longevity (<2 yrs, 2–5 yrs, and >5 yrs) and reproductive technique (asexual, broadcast spawner, egg layer/planktonic larvae, brooder/planktonic larvae, and brooder/mini-adults) – have been considered. Each *taxon* in the database was classified for each trait. The frequency of each category was calculated by summing the abundance of each *taxon* exhibiting that category and weighting by the total abundance of the sample. This resulted in a table that showed the distribution of biological traits at each station over the sampling date. For each trait the frequency of different categories was calculated and then transformed according to the arcsin transformation.

2.4. Statistical analysis

For biotic and diversity indices, differences among dates and basins were tested by a 1-way permutational analysis of variance (PERMANOVA; Anderson (2001), McArdle and Anderson (2001)). The temporal pattern of community in relation to trophic structure and biological traits composition was tested by using a PCA (data transformed according to arcsin(x)).

The relationships between biotic indices and diversity and functioning indices were tested by using Spearman's rank correlation analysis, with Statistica 6.

All multivariate and PERMANOVA analyses were performed by using Primer 6 and PERMANOVA+ software package.

3. Results

3.1. Biotic indices

Temporal trend analyses were carried out by using each basin as a spatial unit, partially solving problems related to spatial heterogeneity, in terms of habitat diversity (see Quintino et al., 2006).

The AMBI index shows significant differences through the time for all the three basins of the lagoon (Table 3), with the highest values recorded in the Northern and

Table 3
Results of PERMANOVA on temporal trends of biotic and functioning indices for each basin; (all replicates, $\log(x+1)$ transformed data)

		Source	df	SS	MS	Pseudo-*F*	*P*(perm)
Northern	AMBI	Date	4	14174	3543.5	19.987	0.001
		Res	144	25530	177.29		
		Total	148	39703			
	Bentix	Date	4	2370.1	592.52	9.5932	0.001
		Res	144	8894.1	61.764		
		Total	148	11264			
	BOPA	Date	4	66290	16572	4.1262	0.001
		Res	144	5.78E+05	4016.4		
		Total	148	6.45E+05			
	Δ rel_exergy	Date	3	575.39	191.8	52.412	0.001
		Res	512	1873.6	3.6594		
		Total	515	2449			
Central	AMBI	Date	5	7786.8	1557.4	8.3824	0.001
		Res	119	22109	185.79		
		Total	124	29895			
	Bentix	Date	5	4042.8	808.56	3.9517	0.005
		Res	119	24348	204.61		
		Total	124	28391			
	BOPA	Date	5	59030	11806	2.9332	0.001
		Res	117	4.71E+05	4024.9		
		Total	122	5.30E+05			
	Δ rel_exergy	Date	4	149.41	37.352	14.463	0.001
		Res	251	648.22	2.5826		
		Total	255	797.63			
Southern	AMBI	Date	4	22191	5547.7	8.7797	0.001
		Res	289	1.83E+05	631.88		
		Total	293	2.05E+05			
	Bentix	Date	4	7337.6	1834.4	6.1885	0.002
		Res	289	85665	296.42		
		Total	293	93003			
	BOPA	Date	4	28290	7072.5	1.4914	0.004
		Res	221	1.05E+06	4742.1		
		Total	225	1.08E+06			
	Δ rel_exergy	Date	3	349.91	116.64	15.951	0.001
		Res	472	3451.4	7.3123		
		Total	475	3801.3			

Central basin in 1988, and in 1995 in the Southern basin (Fig. 2). In general terms, the Southern basin shows a more stable pattern. The comparison among basins shows significant differences for all of them (Table 4).

The temporal pattern of the Bentix index results to be similar to that described for the AMBI, although inverted due to the index formulation (Table 3), with lowest values recorded in 1988 for the Northern and Central basin, and in 1995 for the Southern one (Fig. 3). The main difference from AMBI is the high rating of 1995 and 1999 Central basin samples (Fig. 3). Significant differences are recorded in the comparison between the Northern and the other two basins (Table 4).

The BOPA index shows significant variations during the time (Table 3), with the highest values recorded in 1988 in the Northern and Central basin (Fig. 4) and the lowest ones referred to 1935 samples for all the three basins, 1995 for the Central and Southern basin, and 2001 again for the Central basin. The comparison among the basins

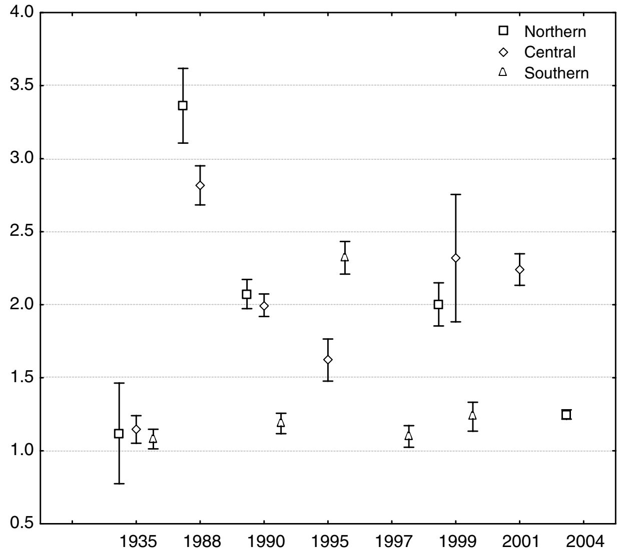

Fig. 2. Temporal trend of the AMBI index.

Table 4
Results of PERMANOVA on temporal trends of biotic and functioning indices, comparison among basins; (all replicates, log($x + 1$) transformed data)

	Source	df	SS	MS	Pseudo-*F*	*P*(perm)	Significant pairwise comp.
AMBI	Basin	2	39250	19625	40.409	0.001	N–C, N–S
	Res	565	2.74E+05	485.67			C–S
	Total	567	3.14E+05				
Bentix	Basin	2	1352.5	676.26	2.8802	0.006	N–C, N–S
	Res	565	1.33E+05	234.79			
	Total	567	1.34E+05				
BOPA	Basin	2	26931	13466	2.9631	0.001	N–S, C–S
	Res	496	2.25E+06	4544.4			
	Total	498	2.28E+06				
Δ rel_exergy	Basin	2	4739.2	2369.6	9.0928	0.001	N–C, C–S
	Res	1245	3.24E+05	260.6			
	Total	1247	3.29E+05				

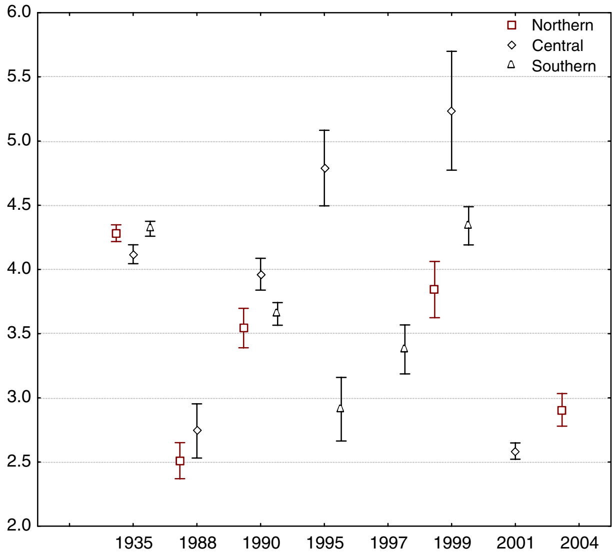

Fig. 3. Temporal trend of the Bentix index.

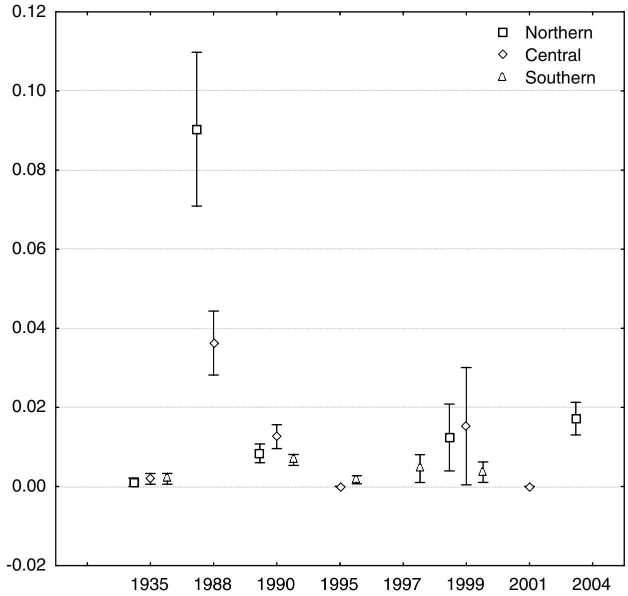

Fig. 4. Temporal trend of the BOPA index.

shows significant differences between the Southern basin and the others (Table 4).

On the basis of the EcoQ ranges proposed for the biotic indices here applied (see Table 2), results obtained for different samples collected in the lagoon of Venice have been classified for each index (Table 5).

3.2. Diversity indices

The Shannon index shows significant temporal trends for all the three basins (Table 6), with a sharp decrease from 1935 to the rest of the series. The lowest values are referred to the Southern and Central basin in 1995 and 1999, respectively (Fig. 5). No significant differences have been detected among the three basins. The classification obtained according to EcoQ ranges is reported in Table 5.

The taxonomic diversity, expressed by the Δ+ index, shows a significant decrease from 1935 to the rest of the series for all the three basins (Table 6), with the lowest values all recorded in the Central basin (Fig. 6). In the comparison among basins, a significant difference is detected between the Central and Southern basin (Table 7).

3.3. Benthic community functioning

To evaluate the functioning of the macrobenthic community, the relative Exergy was applied. In this case the specific relative exergy was expressed in terms of difference between the value of each sample and the 1935 value, used as reference. The temporal trend (significant for all the three basins, see Table 4) shows a negative peak in 1988 for both the Northern and the Central basin, and then a gradual increase peaking in 1997 for the Southern basin (Fig. 7). In 1995 and 1999 two other negative peaks have been recorded, respectively, in the Southern and Central basin.

The analysis of the trophic structure shows both spatial and temporal variations (Fig. 8). The Northern basin seems to be mainly characterized by omnivorous species during

Table 5
Classification of different temporal situations recorded in the three basins of the lagoon of Venice, according to ranges reported in the Table 6 and the WFD status.

	Time	AMBI	Bentix	BOPA	H'	mAMBI
Northern	1935	1.119	4.282	0.001	2.126	0.97198
	1988	3.363	2.510	0.090	1.360	0.72313
	1990	2.073	3.544	0.008	1.521	0.8069
	1999	2.003	3.843	0.012	1.505	0.84895
	2004	1.249	2.906	0.017	1.849	0.90441
Central	1935	1.146	4.119	0.002	2.653	0.99196
	1988	2.817	2.742	0.036	1.321	0.78948
	1990	1.996	3.963	0.013	1.620	0.81085
	1995	1.621	4.790	0.000	1.831	0.79237
	1999	2.319	5.237	0.015	1.074	0.56687
	2001	2.241	2.585	0.000	1.985	0.67613
Southern	1935	1.081	4.317	0.002	2.677	0.93638
	1990	1.188	3.654	0.007	1.736	0.93906
	1995	2.322	2.912	0.002	1.243	0.80014
	1997	1.099	3.478	0.005	1.655	0.93157
	1999	1.230	4.340	0.004	1.745	0.68831

Table 6
Results of PERMANOVA on temporal trends of diversity indices for each basin; (all replicates, $\log(x+1)$ transformed data)

		Source	df	SS	MS	Pseudo-*F*	*P*(perm)
Northern	H′	Date	4	2898.1	724.53	4.7956	0.002
		Res	144	21756	151.08		
		Total	148	24654			
	Δ+	Date	4	7.8253	1.9563	2.5097	0.049
		Res	144	112.25	0.77949		
		Total	148	120.07			
Central	H′	Date	5	8008.7	1601.7	5.2415	0.001
		Res	118	36059	305.59		
		Total	123	44068			
	Δ+	Date	5	677.16	135.43	0.82083	0.425
		Res	116	19139	164.99		
		Total	121	19817			
Southern	H′	Date	4	10022	2505.6	13.349	0.001
		Res	213	39981	187.7		
		Total	217	50003			
	Δ+	Date	4	15.056	3.764	5.4817	0.003
		Res	196	134.58	0.68665		
		Total	200	149.64			

the entire time series, with the exception of 1988 samples dominated by detritus feeders. The Central basin shows major differences through the time, with a structure characterized by omnivores in 1935, and a clear shift towards a filter feeders dominated system in the 90s. Finally, the Southern basin shows variations between a trophic structure characterized by omnivores/predators to one characterized by herbivorous species.

In terms of life history, it is possible to identify three different 'strategies' (Fig. 9). The first one, characterized by species with a medium life span (2–5 yrs) and a 'brooder/mini-adults' reproductive strategy, recorded in 1935 samples for all the basins and other samples of the Southern basin; the second, characterized by species with a short life span (<2 yrs) and an 'egg layer/planktonic larvae' reproductive strategy, referred to 1988 samples; and finally, 'broadcast spawner' species with long life span (>5 yrs) characterizing the '90s samples, mainly in the Central basin.

Although in a context of different patterns (Fig. 10), all biotic indices generally showed a significant correlation (Spearman test) with diversity indices (with the exception

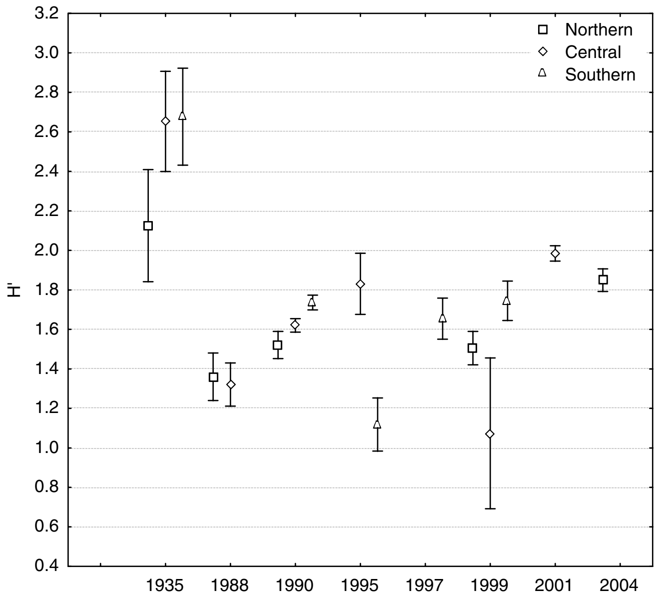

Fig. 5. Temporal trend of the Shannon index.

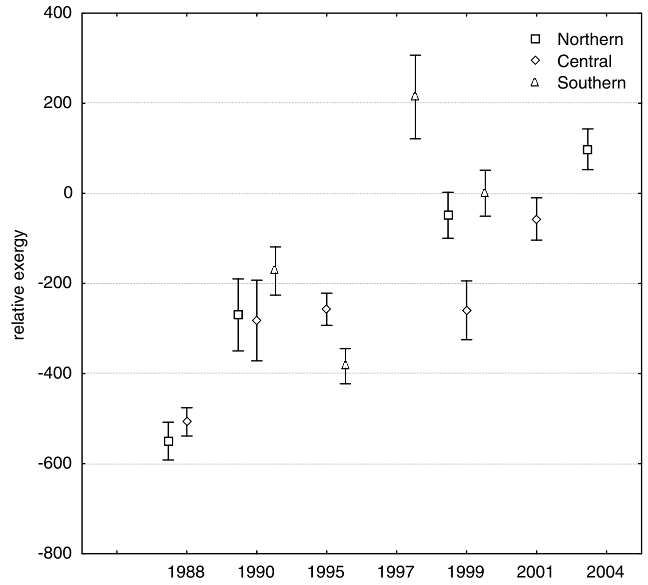

Fig. 7. Temporal trend of relative exergy, expressed using 1935 as reference value.

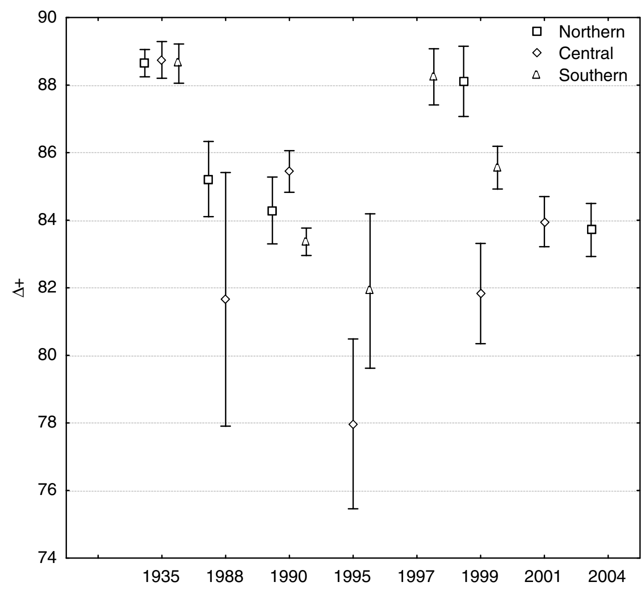

Fig. 6. Temporal trend of Δ+.

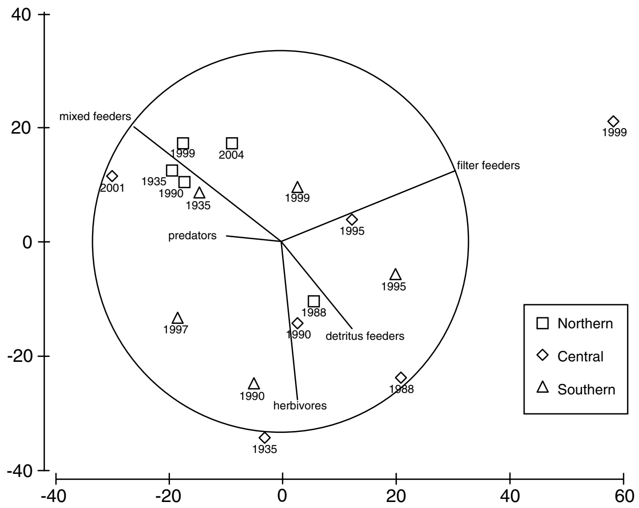

Fig. 8. PCA on trophic structure.

of the Δ+ index for AMBI and Bentix) and with relative exergy and some trophic groups, such as detritus feeders, filter feeders and herbivores).

4. Discussion

In lagoon environments, due to the high sediment surface to water volume ratio, processes occurring within the sediment and at the water-sediment interface strongly

Table 7
Results of PERMANOVA on temporal trends of diversity indices, comparison among basins; (all replicates, $\log(x+1)$ transformed data)

	Source	df	SS	MS	Pseudo-*F*	*P*(perm)	Significant pairwise comp.
H′	Basins	2	498.98	249.49	1.0255	n.s.	–
	Res	488	1.19E+05	243.29			
	Total	490	1.19E+05				
Δ+	Basins	2	184.12	92.059	2.1495	0.021	C–S
	Res	469	20086	42.828			
	Total	471	20270				

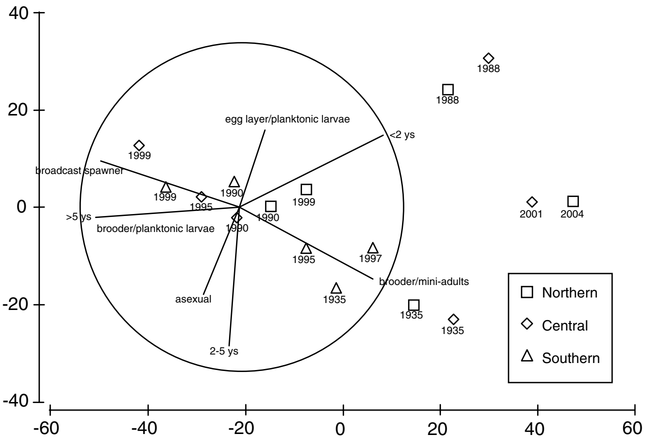

Fig. 9. PCA on life history biological traits.

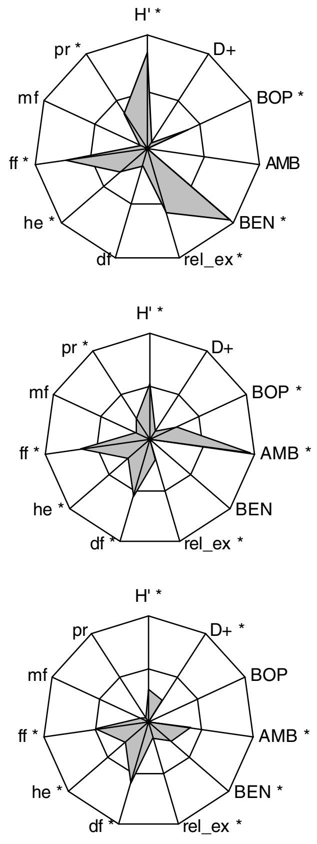

Fig. 10. Relationships between biotic indices and ecological features of macrobenthic community, expressed as absolute value of Spearman's rank correlation coefficients; (d = Margalef index, J = evenness index, H' = Shannon index, $D+$ = Delta index, BOP = BOPA, AMB = AMBI, BEN = Bentix, rel_ex = relative exergy, df = detritus feeders, he = herbivores, ff = filter feeders, mf = mixed feeders, pr = predators; $*$ = statistically significant correlation).

influence the ecosystem metabolism and the nutrient budget (Castel et al., 1996). Therefore, in relation to the fundamental ecological role played by macrobenthic marine invertebrates, and the opportunity to have well-defined responses to environmental changes, the macrobenthic community have attained a crucial role in estuarine and marine environments impact assessment and management (McLusky and Elliott, 2004; Quintino et al., 2006). According to Gibson et al. (2000) metrics/indicators useful in the ecological assessment are those that will discriminate between good and poor quality conditions. Discriminatory ability of indicators can be evaluated by comparing results of each indicator applied to a set of reference sites with the results of indicators from a set of 'known' stressed sites (Gibson et al., 2000; Quintino et al., 2006). In the present study a partially modified version of this approach has been used, comparing results obtained from situations for which the main driving forces acting within the system are known.

It is worth noting that the general picture rising from the biotic indices-based classification is, on average, of 'good' – 'high' conditions for many of the samples collected in all the three basins. This result would be in contrast with the hypothesis of the 'paradox of estuarine quality' (in Dauvin, 2007) suggesting that, being biotic indices based on abundance of stress-tolerant species, and being transitional waters characterized by highly selective conditions, these environments achieve, by definition, low scores and so low EcoQ values (on this topic see also Simboura, 2004).

This result could be partially explained by the peculiarity of the lagoon of Venice, characterised by a high morphological heterogeneity with a variety of different habitats and gradients, resulting in less pronounced scarcity of species, typical of other transitional environments, but also highlights the importance to revise and redefine ranges of refence on an European scale, taking into the consideration the peculiarities of different geographical areas.

In general terms, Bentix seems to be the index with the highest resolving power, resulting in a better discrimination among samples, whereas BOPA, on the other hand, shows a low discrimination, classifying all the samples in the 'high' category, with the exception of one, the Northern basin in 1988, classified as 'good'.

The comparison between AMBI and Bentix results, in a context of general concordance of the patterns, highlights some differences. Indeed, both the indices classify as 'high' the 1935 samples for all the three basins, and the 1988 in the Northern basin as 'moderate'. For AMBI all other samples, except the Southern basin in 1997, classified as 'high', resulted 'good'; whereas, according to Bentix there are other samples classified as 'moderate' (the Central basin in 1988 and 2001, and the Southern basin in 1995). Another discrepancy between the two indices is in relation to the classification of samples collected in the Central basin in 1995 and 1999, classified 'high' by Bentix and 'good' by AMBI (see Table 5). According to Simboura and Reizopoulou (2007), this could be related to the different design of each index (in Bentix each ecological group is weighted equally, whereas AMBI renders a different coefficient for each one; moreover the scaling of the distances among classes is different in the two methods).

The Bentix index seems, therefore, to be more sensitive than AMBI to increases of the organic matter content in the bottom sediments and related changes in macrobenthic assemblage, see the low score – 'moderate' classification – achieved by the 1988 samples for both the Northern and Central basin, during the macroalgae blooms phase (Sfriso et al., 2003), dominated by high flows of energy and matter towards the sediment, and characterized by the high incidence of detritus feeders trophic group. On the opposite, this produced a high rating of the samples collected in the central basin in 1999, even if characterized by a high mechanical clam harvesting pressure, which deeply affect both sediment texture, with loss of the fine fraction (Pranovi et al., 2004) and a benthic community overdominated by the hard shelled species, mainly Manila clams (Pranovi et al., 2006).

The results obtained in the present study are consistent with those published by other authors (Simboura, 2004; Occhipinti Ambrogi et al., 2005; Dauvin et al., 2007), confirming, on one hand, discrepancies between AMBI and Bentix classifications, and on the other that the Bentix index seems to be, in some circumstances, better discriminating than AMBI.

The 'paradox of estuarine quality' could, instead, be useful to explain the Shannon index results, which shows an intermediate resolving power. Indeed, almost all samples are classified 'poor', with the exception of the 1988 samples from the Northern and Central basin, the 1995 one from the Southern basin and the 1999 one from the Central basin, classified 'bad'. The pattern directly reflects that of diversity (see $\Delta+$ results) combined with the dominance of few species in some critical periods (such as in 1988 and 1999).

The multimetric approach suggested by m-AMBI, combining AMBI, H′ and S, results in a poor discriminating power, producing the same pattern recorded for AMBI.

A summary of the main ecological features and biotic indices–based classification for the four main states recorded in the lagoon during the last 70 years is reported in Table 8. Along with the change in the main driving forces, the macrobenthic community showed significant differences both in terms of structure and functioning. The classification obtained by applying different biotic indices cannot resolve all differences recorded in terms of community features. To similar EcoQ values can correspond quite different community features, which directly reflect in different functioning of the benthic compartment.

In accordance with observations reported for other not strictly marine ecosystems (see Labrune et al., 2006), biotic indices seem to generally overestimate the Ecological Quality Status (EcoQ) under different conditions, for which diversity and functioning indices show low values, as well as deep changes in life history traits (Table 8).

A general concordance between biotic and structure/functioning indices has been registered for the 1935, which represents a sort of 'pristine state', characterized by high diversity (both in terms of number of species and taxonomic diversity) and a mature condition, with a trophic structure dominated by epibenthic omnivorous species.

Discrepancies have been instead recorded for the other three states, which represent bad ecological conditions, but scored high biotic indices values.

In 1988 the lagoon experienced a dystrophic phase, being this phenomenon recognized as one of the most catastrophic symptoms of benthic community degeneration (Valiela et al., 1997). During this period, the huge macroalgae biomass caused a confinement of a great amount of energy, normally flowing through the ecosystem, in a single compartment, and greatly increased fluxes towards the bottom sediment. All this reflected in an extremely low value of transfer efficiency within the trophic web (Libralato et al., 2006), and a benthic community dominated by opportunistic (short life-span with planktonic larvae), detritus feeder species, showing low system maturity.

In 1995, the Southern basin faced a degradation due to eutrophication and chemical pollution (Sorokin et al., 2002), with benthic community showing modifications similar to those previously described, such as a trophic structure dominated by detritus feeders, which is a sign of decrease of the ecological quality (Mearns and Word, 1982).

Finally in 1999, the overdominance of Manila clam and the heavy pressure of mechanical harvesting for clams, deeply affected the benthic compartment of the Central basin (Pranovi et al., 2004, 2006). The macrobenthic community shifted towards infaunal, filter feeder species, characterized by low mobility and low fragility, again with a low system maturity.

Table 8
Main features of the benthic community referred to the three main ecological stages, and biotic indices classification (A/B = AMBI/Bentix)

	1935 (all the three basins)	1988 (Northern and Central basin)	1995 (Southern basin)	1999 (Central basin)
Driving force	Scarce anthropogenic pressures	Eutrophication/macroalgae blooms	Degradation by pollution	Manila clam and its exploitation
Diversity	High	Low	Low	Medium
Δ rel_exergy	Medium/high	Low	Low	Medium
Dominant trophic group	Mixed feeders	Detritus feeders	Detritus feeders	Filter feeders
Life history traits	Medium life (2–5 yrs) Brooder/mini-adults	Short life (<2 yrs) Egg layer/planktonic larvae	Medium life (2–5 yrs) Brooder/mini-adults	Long life (>5 yrs) Broadcast spawner
Bioic indices classification (A/B)	High/high	Good/moderate	Good/moderate	Good/high

On the basis of the results obtained in the present study, some preliminary conclusions can be proposed.

First of all, the difficulty related to the use of a biotic index in environments subjected simultaneously to many different kinds of stressors, or the use of indices against stressors for which the indicators were not originally developed (Quintino et al., 2006).

The confirmation of the need to reach to a consensus regarding at least the most important species scores applying for all different European areas, as suggested by JRC-EEWAI (2007).

The rising of the question about the identification of units, in terms of typical habitats and/or homogeneous areas, on which to base the evaluation of the ecological status. In the present study, the treatment of the results per 'basin' unit, would reduce interference due to the effects of environmental gradients such as salinity and sediment type, which influence biotic indices (Llanso et al., 2002), and probably smooth the effects (Quintino et al., 2006), whereas significant differences among the three basins have been recorded. This could be related, for example, to a different composition in terms of habitats, which reflects in different resilience of each basin to external pressures (such as the potential buffering role played by seagrass meadows in the Southern basins, see Duffy, 2006), but also to a different spatial distribution of the main ecological drivers. All these can directly affect the choice of reference values/states within morphologically complex environments, such as coastal lagoons.

Possible solutions could be related to the application of a multimetric approach (but see results obtained by m-AMBI), combining together different metrics, such as biotic and diversity indices (Magni et al., 2005; Dauvin et al., 2007; Quintino et al., 2006), and/or the intercalibration process, as proposed by Borja et al. (2007).

On the other hand, however, it would be necessary to implement the use of functioning indicators, improving the knowledge about the relationships between structure and functioning at community/ecosystem level (Raffaelli, 2006). In this context, obtained results confirm those reported by Marques et al. (2003) about the utility of relative exergy as an indicator able to capture the state of the system and distinguish between different scenarios.

Acknowledgements

We thank Dr. Argyro Zenetos for the constructive comments on the manuscript. This work was supported by a CNR grant, in the context of the Short Term Mobility Program 06.

References

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26, 32–46.

Bendoricchio, G., Jørgensen, S.E., 1997. Exergy as goal function of ecosystem dynamics. Ecological Modelling 102, 5–15.

Borja, A., Heinrich, H., 2005. Implementing the European water framework directive: the debate continues. Marine Pollution Bulletin 50, 486–488.

Borja, A., Franco, J., Perez, V., 2000. A marine biotic index to the establish ecology quality of soft-bottom benthos within European estuarine coastal environments. Marine Pollution Bulletin 40, 1100–1114.

Borja, A., Muxika, I., Franco, J., 2003. The application of a marine biotic index to different impact sources affecting soft-bottom benthic communities along the European coasts. Marine Pollution Bulletin 46, 835–845.

Borja, A., Franco, J., Muxika, I., 2004a. The biotic indices and the water framework directive: the required consensus in the new benthic monitoring tools. Marine Pollution Bulletin 48, 405–408.

Borja, A., Franco, J., Valencia, V., Bald, J., Muxika, I., Belzune, M.J., Solaun, O., 2004b. Implementation of the European water framework directive from the Basque country (northern Spain): a methodological approach. Marine Pollution Bulletin 48, 209–218.

Borja, A., Josefson, A.B., Miles, A., Muxika, I., Olsgard, F., Phillips, G., Rodríguez, J.G., Rygg, B., 2007. An approach to the intercalibration of benthic ecological status assessment in the North Atlantic ecoregion, according to the European water framework directive. Marine Pollution Bulletin 55, 42–52.

Castel, J., Caumette, P., Herbert, R., 1996. Eutrophication gradients in coastal lagoons as exemplified by the Bassin d'Arcachon and the Etang du Prèvost. Hydrobiologia 329, 9–28.

Cesari, P., Pellizzato, M., 1985. Molluschi pervenuti in Laguna di Venezia per apporti volontari o casuali Acclimatazione di *Saccostrea commercialis* (Iredale and Roughely, 1933) e di *Ruditapes philippinarum* (Adams & Reeve, 1850). Bollettino Malacologico 21, 237–274.

Clarke, K.R., Warwick, R.M., 2001. A further biodiversity index applicable to species lists: variation in taxonomic distinctness. Marine Ecology Progress Series 216, 265–278.

Cossu, R., De Fraja Frangipane, E., 1985. Stato delle conoscenze sull'inquinamento della laguna di Venezia. Ministro dei Lavori Pubblici – Magistrato alle acque, vols. I–IV. Consorzio Venezia Nuova, Venice, Italy, 438 pp.

Dauvin, J.-C., 2007. Paradox of estuarine quality: Benthic indicators and indices, consensus or debate for the future. Marine Pollution Bulletin 55, 271–281.

Dauvin, J.-C., Ruellet, T., 2007. Polychaete/amphipod ratio revisited. Marine Pollution Bulletin 55, 215–224.

Dauvin, J.-C., Ruellet, T., Desroy, N., Janson, A., 2007. The ecological quality status of the Bay of Seine and the Seine estuary: use of biotic indices. Marine Pollution Bulletin 55, 241–257.

Debeljak, M., 2002. Applicability of genome size in exergy calculation. Ecological Modelling 152, 103–107.

Desrosiers, G., Bellan-Santini, D., Brêthes, J.C., 1986. Organisation trophique de quatre peuplements de substrats rocheux selon un gradient de polution industrielle (Golfe de Fos, France). Marine Biology 91, 107–120.

Desrosiers, G., Savenkoff, C., Olivier, M., Stora, G., Juniper, K., Caron, A., Gagnè, J-P., Legendre, L., Mulsow, S., Grant, J., Roy, S., Grehan, A., Scaps, P., Silverberg, N., Klein, B., Tremblay, J-E., Therriault, J-C., 2000. Trophic structure of macrobenthos in the Gulf of St. Lawrence and on the Scotian Shelf. Deep-Sea Research 47, 663–697.

Duffy, J.E., 2006. Biodiversity and functioning of seagrass ecosystems. Marine Ecology Progress Series 311, 233–250.

EC, 2000. Directive of the European parliament and of the Council 2000/60/EC establishing the framework for community action in the field of the Water Policy. PE-CONS 3639/1/00.

Fauchauld, K., Jumars, P.A., 1979. The diet of worms: a study of polychaete feeding guilds. Oceanograph and Marine Biology: Annual Review 17, 193–284.

Fonseca, J.C., Marques, J.C., Paiva, A.A., Freitas, A.M., Madeira, V.M.C., Jørgensen, S.E., 2000. Nuclear DNA in the determination of weighing factors to estimate exergy from organisms biomass. Ecological Modelling 126, 179–189.

Gibson, G.R., Bowman, M.L., Gerritsen, J., Snyder, B.D., 2000. Estuaries and Coastal Marine Waters: Bioassessment and Biocriteria Technical Guidance. EPA 882-B-00-024. U.S. Environmental Protection Agency, Office of Water, Washington, DC. <www.epa.gov/owow/monitoring/>.

Gomez Gesteira, L., Dauvin, J.C., 2000. Amphipods are good bioindicators of the impact of oil spills on soft-bottom macrobenthic communities. Marine Pollution Bulletin 40, 1017–1027.

Grall, J., Glémarec, M., 1997. Using biotic indices to estimate macrobenthic community perturbations in the Bay of Brest. Estuarine and Coastal Shelf Science 44, 43–53.

Gregory, T.R., 2007. Animal genome size database. <http://www.genomesize.com>.

Jørgensen, S.E., Nielsen, S.N., Mejer, H., 1995. Emergy, environ, exergy and ecological modelling. Ecological Modelling 77, 99–109.

JRC-EEWAI, 2007. Intercalibration technical report. Part 3 – Coastal and Transitional Waters, Section 2 – Benthic Invertebrates. 130 pp.

Labrune, C., Amouroux, J.M., Sarda, R., Dutrieux, E., Thorin, S., Rosenberg, R., Grémare, A., 2006. Characterization of the ecological quality of the coastal Gulf of Lions (NW Mediterranean). A comparative approach based on three biotic indices. Marine Pollution Bulletin 52, 34–47.

Llanso, R.J., Scott, L.C., Dauer, D.M., Hyland, J.L., Russell, D.E., 2002. An estuarine benthic index of biotic integrity for the Mid-Atlantic region 'of the United States. I. Classification of assemblages and habitat definition. Estuaries 25, 1219–1230.

Libralato, S., Torricelli, P., Pranovi, F., 2006. Exergy as ecosystem indicator: an application to the recovery process of marine benthic communities. Ecological Modelling 192, 571–585.

Magni, P., Hyland, J., Manzella, G., Rumohr, H., Viaroli, P., Zenetos, A. (Eds.), 2005. Proceedings of the Workshop ''Indicators of Stress in the Marine Benthos. IOC Workshop Reports, vol. 195, 46 pp.

Marques, J.C., Jørgensen, S.E., 2002. Three selected ecological observations interpreted in terms of a thermodynamic hypothesis. Contribution to a general theoretical framework. Ecological Modelling 58, 1–9.

Marques, J.C., Pardal, M.A., Nielsen, S.N., Jørgensen, S.E., 1997. Analysis of the properties of exergy and biodiversity along an estuarine gradient of eutrophication. Ecological Modelling 102, 155–167.

Marques, J.C., Nielsen, S.N., Pardal, M.A., Jørgensen, S.E., 2003. Impact of eutrophication and river management within a framework of ecosystem theories. Ecological Modelling 166, 147–168.

McArdle, B.H., Anderson, M.J., 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. Ecology 82, 290–297.

McLusky, D.S., Elliott, M., 2004. The Estuarine Ecosystem; Ecology, Threats and Management, third ed. Oxford University Press, Oxford, p. 216.

Mearns, A.J., Word, J.Q., 1982. Forecasting effects of sewage solids on marine benthic communities. In: Mayer, G.F. (Ed.), Ecological Stress and the New York Bight: Science and Management. Estuarine Research Federation, Columbia, pp. 495–512.

Muxika, I., Borja, A., Bonne, W., 2005. The suitability of the marine biotic index (AMBI) to new impact sources along European coasts. Ecological Indicators 5, 19–31.

Muxika, I., Borja, A., Bonne, W., 2007. Using historical data, expert judgement and multivariate analysis in assessing reference conditions and benthic ecological status, according to the European Water Framework Directive. Marine Pollution Bulletin 55, 16–29.

Occhipinti-Ambrogi, A., Forni, G., Marchini, A., 2005. Testing Different Approaches for Quality Assessment Using the Benthic Community: Examples from the Northern Adriatic Sea. In: Magni, P., Hyland, J., Manzella, G., Rumohr, H., Viaroli, P., Zenetos, A. (Eds.), Proceedings of the Workshop ''Indicators of Stress in the Marine Benthos, IOC Workshop Reports, vol. 195, pp. 23–26.

Odum, E.P., 1969. The strategy of ecosystem development. Science 164, 262–270.

Pearson, T., Rosenberg, R., 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology: Annual Review 16, 229–311.

Pranovi, F., Da Ponte, F., Raicevich, S., Giovanardi, O., 2004. A multidisciplinary study of the immediate effects of mechanical clam-harvesting in the Venice Lagoon. ICES Journal of Marine Science 61, 43–52.

Pranovi, F., Franceschini, G., Casale, M., Zucchetta, M., Giovanardi, O., Torricelli, P., 2006. An ecological imbalance induced by a non-native species: the Manila clam in the Venice Lagoon. Biological Invasions 8, 595–609.

Pranovi, et al., submitted for publication. Historical changes in the structure and functioning of the benthic community in the lagoon of Venice. Estuarine and Coastal Shelf Science.

Provincia di Venezia, 2000. Piano per la gestione delle risorse alieutiche delle lagune della provincia di Venezia. Provincia di Venezia, pp. 102.

Quintino, V., Elliott, M., Rodrigues, A.M., 2006. The derivation, performance and role of univariate and multivariate indicators of benthic change: case studies at differing spatial scales. Journal of Experimental Marine Biology and Ecology 330, 368–382.

Raffaelli, D.G., 2006. Biodiversity and ecosystem functioning: issues of scale and trophic complexity. Marine Ecology Progress Series 311, 285–294.

Ravera, O., 2000. The Lagoon of Venice: the result of both natural factors and human influence. Journal of Limnology 59, 19–30.

Sfriso, A., Facca, C., Ghetti, P.F., 2003. Temporal and spatial changes of macroalgae and phytoplankton in a Mediterranean coastal area: the Venice Lagoon as case study. Marine Environmental Research 56, 316–636.

Simboura, N., 2004. Bentix index vs biotic index in monitoring: an answer to Borja et al., 2003. Marine Pollution Bulletin 48, 403–404.

Simboura, N., Zenetos, A., 2002. Benthic indicators to use in ecological quality classification of Mediterranean soft bottom marine ecosystems, including a new biotic index. Mediterranean Marine Science 3, 77–111.

Simboura, N., Reizopoulou, S., 2007. A comparative approach of assessing ecological status in two coastal areas of Eastern Mediterranean. Ecological Indicators 7, 455–468.

Simboura, N., Orfanides, S., Zenetos, A., 2005. Ecological status and trends. In: Papathanassiou, V., Zenetos, A. (Eds.), SoHelME. State of the Hellenic Marine Environment. HCMR Publ., pp. 343–351, pp. 360.

Sorokin, P., Sorokin, Y., Zakuskina, O., Ravagnan, G., 2002. On the changing ecology of Venice lagoon. Hydrobiologia 487, 1–18.

Svirezhev, Yu.M., 2000. Thermodynamics and ecology. Ecological Modelling 132, 11–22.

Todd, J., 2006. <http://eusmilia.geology.uiowa.edu/database/bivalves/Bivalve_eco.html>.

Tumbiolo, M.L., Downing, J., 1994. An empirical model for the prediction of secondary production in marine benthic invertebrate populations. Marine Ecology Progress Series 114, 165–174.

UNEP/MAP, 2005. Fact sheets on Marine pollution indicators. Document UNEP(DEC)/MED/WG.264/Inf.14, 254 pp.

Valiela, I., McClelland, J., Hauxwell, J., Behr, P.J., Hersh, D., Foreman, K., 1997. Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. Limnology and Oceanography 42, 1105–1118.

Wall, G., 1977. Exergy – a useful concept within resource accounting Report No. 77–42. Institute of Theoretical Physics, Chalmers University of Technology and University of Goteborg, Goteborg, Sweden, 58pp.