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Research paper

Benthic foraminifera to assess Ecological Quality Statuses in Italian transitional waters

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ABSTRACT

Estuaries, lagoons and lakes, identified as transitional waters (TWs), are fragile ecotones at the interface between terrestrial and marine ecosystems. The assessment of their Ecological Quality status (EcoQ), required by marine legislations, is rather uncertain when based on traditional benthic macrofaunal indices. This study proposes to assess the EcoQ of marine waters using the diversity index Exp(H'_{bc}) based on living benthic foraminifera. By testing this method on datasets from five Italian TWs, we showed that EcoQ's assessment based on foraminifera reflected changes in environmental conditions. Benthic foraminifera highlighted the degradation of most, if not all, study sites, ranking most of the TWs in "Moderate" to "Bad" EcoQs. Although the assessment of EcoQs estimated from diversity indices was similar when based on benthic foraminifera or macrofauna, discrepancies occurred if diversity index calculated on foraminifera and sensitivity-based indices applied on macrofauna were compared. Our results demonstrated that the Exp(H'_{bc}) index based on living benthic foraminifera is a promising approach to assess EcoQs. Finally, we argue that benthic foraminifera, for their preservation potential as fossil within sediment, are a reliable option to define reference conditions and targets.

1. Introduction

Lagoons, coastal lakes and estuaries, commonly identified as transitional waters (hereafter TWs; McLusky and Elliott, 2007), are located at the interface between sea and land. Under the influence of both marine and freshwaters, these fragile ecotones are characterised by strong environmental gradients and high variability of physico-chemical parameters (*e.g.* salinity, temperature, oxygen; Elliott and Quintino, 2007). In addition, they are naturally enriched in organic matter (Pusceddu et al., 2003). Transitional waters therefore appear as naturally stressed environments (Elliott and Quintino, 2007) much more selective than classic terrestrial ecotones. Representing at least 13% of the world's coastline (Nixon, 1982), they provide key ecosystems services such as habitat and food resources for migratory birds (Munari and Mistri, 2012) and nursery habitats for juvenile fish species of high commercial value (*e.g.* flatfishes, eels; Beck et al., 2001; Couturier et al., 2007). They are, however, exposed to high levels of anthropogenic stresses such as aquaculture, habitat destruction, urban sewage and industrial activities (Bouchet and Sauriau, 2008; Frontalini et al., 2009; Francescangeli et al., 2016).

In order to protect and restore these fragile ecosystems, marine legislations worldwide, *i.e.* the US Clean Water Act, the EU Water Framework Directive (WFD, 2000/60/EC), the Marine Strategy Framework Directive (MSFD, 2008/56/EC) and the National Water Act in South Africa, emphasised the need to assess the health of TWs (Munari and Mistri 2008). A plethora of methodologies and tools, based on benthic macrofauna (Borja et al., 2000), seagrasses (Krause-Jensen et al., 2005), fishes (Coates et al., 2007) and, more recently, on benthic foraminifera (Bouchet et al., 2012), have been suggested in this sense; benthic macrofauna being the most widely used to assess Ecological Quality status (EcoQ) in marine environments (Dauvin et al., 2012). Most macrofaunal indices rely on the response of species towards an enrichment in organic matter (OM) in sediment (Borja et al., 2000; Bouchet and Sauriau, 2008; Lavesque et al., 2009). In TWs, OM content in sediment is naturally high, promoting tolerant to opportunistic species, while sensitive species decline (Elliott and Quintino, 2007; Munari and Mistri, 2008). Because of these natural features, benthic communities, such as those observed in Italian lagoons and coastal lakes, are hence similar to those found in anthropogenically disturbed areas (Munari and Mistri, 2008). Ecological Quality statuses of most TWs would be inaccurately determined as "Poor" or "Bad" (Elliott and Quintino, 2007; Munari and Mistri, 2008). Therefore, EcoQ assessment

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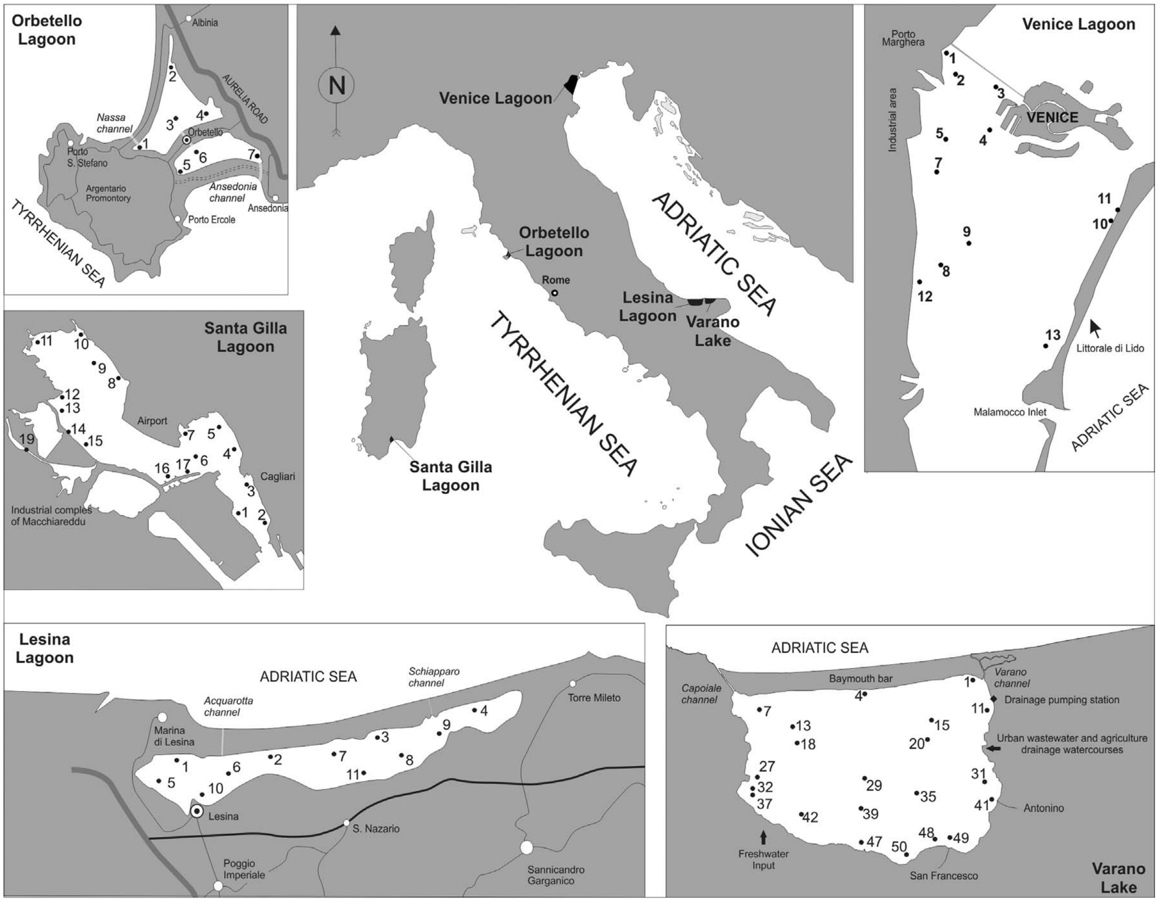

Fig. 1. Localisation of the five Italian transitional waters sites and sampling stations. For each study site (Orbetello, Santa Gilla, Lesina and Venice Lagoons and Varano Lake), main pressures are mentioned (*e.g.* industrial areas, drainage, freshwater inputs).

based on benthic macrofaunal species is not optimal in TWs (Blanchet et al., 2008; Bouchet and Sauriau, 2008; Lavesque et al., 2009), especially in Italian areas (Munari and Mistri, 2008; Munari et al., 2009a; Borja et al., 2011; Munari and Mistri, 2012).

To assess the health of marine systems, it is mandatory to define reference conditions or to set targets according to the WFD and to the MSFD, respectively. Monitoring programs using benthic macrofauna have highlighted the difficulty of defining reference conditions and setting targets in TWs (Teixeira et al., 2008; Borja et al., 2011; Borja et al., 2012). As a consequence, as no reference exists for most areas (*i.e.* a pristine, unpolluted, or anthropogenically undisturbed state; Hughes, 1995; Davies and Jackson, 2006), "good" conditions and targets are commonly based on expert judgment (Muxika et al., 2007). While expert judgment may have some advantages (Muxika et al., 2007), more objective approaches, which imply the elaboration of new methods and indicators, are essential to better evaluate the health of transitional waters.

In coastal and transitional waters, benthic foraminifera are relevant indicators of human-induced stresses (*e.g.* Alve, 1995; Coccioni et al., 2009; Frontalini et al., 2009) such as aquaculture (Bouchet et al., 2007), oil spills (Morvan et al., 2004), trace elements (Armynot du Châtelet et al., 2004) or urban sewage (Burone et al., 2006). Due to their short life cycles, benthic foraminifera respond quickly to both environmental variability and changes in population dynamics and species composition are indicative of changes in environmental conditions (Schönfeld et al., 2012). In addition, as they occur in almost all marine environments and in higher abundances than macrofauna, quantitative data are easily obtained in a small volume of sediment (*i.e.* a few cm^3). Benthic foraminiferal diversity has been successfully used to assess EcoQs in fjords along the Norwegian Skagerrak coast (North Sea; Bouchet et al., 2012), in the Oslofjord (Norway; Dolven et al., 2013) and in the Boulogne-sur-Mer harbour (Northeastern France; Francescangeli et al., 2016). Because of their potential to reconstruct palaeo-environments (Alve, 1991; Hayward et al., 2004; Alve et al., 2009), a unique feature compare to benthic macrofauna (except for molluscs; Poirier et al., 2009), they are also good candidates to help in establishing objective and reliable reference conditions. As a consequence, benthic foraminifera appear to be a relevant complement to benthic macrofauna to assess EcoQs.

Growing evidences support a decline in the biological integrity of Italian TWs over the last decades (Munari and Mistri, 2012). These ecosystems are indeed strongly modified by intense human activities *i.e.* aquaculture, industries, agriculture and urban sewage outfall (Marchini et al., 2008; Coccioni et al., 2009; Lardicci et al., 2001; Munari and Mistri, 2008). Amongst it, Venice, Orbetello, Lesina and Santa Gilla lagoons and Varano lake are particularly affected (Frontalini et al., 2010; Degetto et al., 1997; Munari and Mistri 2008; Borja et al., 2011). As a result, there is an urgent need to develop and test methods which overcome the aforementionned limitations of traditional biotic indices in order to assess the EcoQ of Italian TWs (Munari and Mistri, 2008). Here, we decided to focus on benthic foraminifera as they are likely to add complementary information to commonly used indices. Using existing datasets, we tested the applicability of the method developed by Bouchet et al. (2012), the diversity index $Exp(H'_{bc})$ calculated on benthic foraminifera, to assess changes in the EcoQs of the five aforementioned Italian transitional waters. We also investigated possible correlation between changes in this diversity index and anthropogenic disturbances (*i.e.* organic matter, trace elements). Finally, since benthic macrofauna is traditionally used in environmental monitoring, we compared and discussed, for the five sites, EcoQs obtained with benthic foraminifera and those calculated with benthic macrofauna in previous studies.

2. Materials and methods

2.1. Study sites

Five TWs along the Italian coast were considered: Santa Gilla, Venice, Orbetello, Lesina lagoons and Varano Lake with a total of 13, 18, 7, 11 and 21 stations at each site, respectively (Fig. 1). These case studies are based on data and results from the authors and other published sources representing a variety of pollution types, environmental problems and geographic settings. In addition, sampling of foraminifera and macrofauna at all study sites allowed to compare EcoQs obtained with both groups.

2.2. Sedimentological and foraminiferal sampling

Samples (one replicate) were taken in 2006 in Santa Gilla, in 2002 in Venice, in 2004 in Orbetello, in 2004 in Lesina and in 2012 in Varano (Coccioni et al., 2009; Frontalini et al., 2009; Frontalini et al., 2010; Frontalini et al., 2013). The geographical positions of the stations were determined using the Global Position System and samples were collected by means of a Van Veen grab sampler which collects sediment over a surface area of about 400 cm^2. Benthic foraminifera have a well-known patchy distribution with heterogeneous patterns over a few centimetres (Boltovskoy and Lena, 1969). While three replicates have recently been suggested to be an adapted sampling strategy in subtidal areas (Bouchet et al., 2012), one replicate is still often done for survey of benthic foraminiferal communities (*e.g.* Mojtahid et al., 2008; Barras et al., 2014; Nesbitt et al., 2015). Besides, the surface sampled by a Van Veen Grab is fairly higher than the micro-scale variability observed in benthic foraminiferal distribution. As a consequence, a one replicate sampling strategy is representative of the diversity of foraminiferal communities at the study sites.

On board, the grab was immediately and carefully opened in a container, the sediment being deposited in its initial position. For each sample, two aliquots were taken and stored in polyethylene jars: the first was used to measure trace element contents in the sediment (see “2.4 Trace elements analysis”), the second being utilised for a thorough examination of the benthic foraminiferal assemblages (see “2.6 Foraminiferal analysis”). Sediment samples for the study of benthic foraminifera were stained with buffered rose Bengal dye (2 g of rose Bengal in 1000 ml of ethyl alcohol) to distinguish living from dead specimens (see Schönfeld et al., 2012 for the methodology). For each site, comprehensive descriptions of the environmental conditions and benthic foraminiferal communities are available in the literature (Coccioni et al., 2009, Frontalini et al., 2009; Frontalini et al., 2010; Frontalini et al., 2013).

2.3. Grain size analysis

Grain size analysis was performed to determine the percentage of silt and clay (< 63 mm) and the fraction superior to 63 μm was dried and fractionated by ASTM (American Society for Testing and Materials) micro-sieve.

2.4. Trace elements analysis

Samples were dried, reduced to a fine powder and used to determine trace element contents in sediments (*i.e.* Pb, Cu, Cd and Zn). Activation Laboratories Ltd. (Ontario, Canada, http://www.actlabs.com) analysed a fraction of ~0.5 g of a sample for 32 elements. The sample material was digested with aqua regia (0.5 ml H_2O, 0.6 ml concentrated HNO_3 and 1.8 ml concentrated HCl) for 2 h at 95 °C. Samples were then cooled, diluted to 10 ml with de-ionised water, homogenised and analysed using a Perkin Elmer OPTIMA 3000 Radial ICP for the element suite. A standard matrix and a blank were run every 13 samples and a series of USGS-geochemical standards were used as controls.

2.5. Organic carbon contents

Total Organic Carbon (TOC) contents were based on previous studies carried out in Varano Lake (Frontalini et al., 2013) and in Venice (Secco et al., 2005), Lesina (Borja et al., 2011), Orbetello (Specchiulli et al., 2010) and Santa Gilla lagoons (G. Atzori, personal communication).

2.6. Foraminiferal analysis

All samples were dried at 50 °C and weighed. They were then gently washed with tap water through a 63 μm sieve to remove clay, silt and any excess dye and the residual fraction was re-dried at 50 °C and weighed again to determine the mud fraction. Quantitative analysis of benthic foraminifera was performed on the fraction > 63 μm. According to Murray and Bowser (2000), only specimens with dense, brightly red-stained protoplasm were considered as alive. When possible, three hundred stained specimens per sample were picked and identified, following the generic classifications of Loeblich and Tappan (1987).

2.7. Data analyses

2.7.1. Ecological Quality Statuses based on environmental conditions

We evaluated changes in EcoQs based on fluctuations in both trace elements (*i.e.* Pb, Cu, Cd and Zn) and organic matter (TOC) contents using the PERSE procedure (Procedure to Establish a Reference State for Ecosystems; Rombouts et al., 2013), a multivariate non-parametric method that allows the calculation of relative reference states against which fluctuations can be detected and quantified. A detailed mathematical description of this technique is provided in Goberville et al. (2011a,b).

For each station, the technique has been performed to calculate the probability that an observation (*i.e.* the combination of the four trace elements plus the organic matter content) belongs to a theoretical relative reference state determined from the criteria defined by Bakke et al. (2010) using the “Good” EcoQ limit values (Table 1) as references. Such a procedure is an efficient way to resume, in a single value, the multidimensionality of an exhaustive dataset of environmental parameters while taking into consideration the covariance among descriptors (Ibañez, 1981). The resulting probabilities, *i.e.* measures of the distance between each observation and the centroïde of the relative reference state that represent an index of environmental conditions, were retained.

2.7.2. Ecological Quality Statuses based on benthic foraminifera

Following the methodology proposed by Bouchet et al. (2012), EcoQs of the 70 stations from the five sites were determined with the diversity index Exp(H'_{bc}) based on benthic foraminifera. Because TWs

Table 1
Environmental criteria (provided by Bakke et al., 2010) and diversity (Exp(H'_{bc})) values (modified from Bouchet et al., 2012) used to assess the Ecological Quality status of the different stations sampled in the five Italian transitional waters sites.

	Excellent	Good	Moderate	Bad	Poor
Pb (μg/g)	< 30	30–83	83–100	100–720	> 720
Cu (μg/g)	< 35	35–51	51–55	55–220	> 220
Cd (μg/g)	< 0.25	0.25–2.6	2.6–15	15–140	> 140
Zn (μg/g)	< 150	150–360	360–590	590–4500	> 4500
TOC (%)	< 2	2–2.7	2.7–3.4	3.4–4.1	> 4.1
Exp(H'_{bc})	> 15	11–15	7–11	3–7	< 3

Table 2
Ecological Quality Status of the five Italian transitional waters sites assessed using the diversity index Exp(H'$_{bc}$), calculated from benthic foraminifera (this study), and 6 indices (Shannon diversity H', BENTIX, AMBI, BITS, FINE and BOPA; see text for references) based on benthic macrofauna (from Munari and Mistri, 2007; Munari et al., 2009a; Munari et al., 2009b; Specchiulli et al., 2010; Cabiddu et al., 2014). ND: no data available.

	Foraminifera	Macrofauna					
	Exp(H'$_{bc}$)	H'	BENTIX	AMBI	BITS	FINE	BOPA
Varano	Poor to Good	Bad to Poor	ND	ND	ND	ND	ND
Lesina	Bad	Bad to Moderate	Bad to Good	Moderate to Good	Poor to Good	Moderate to High	Good to High
Venice	Bad to Moderate	Poor to Moderate	Bad	Poor to Good	Poor to High	Moderate to High	High
Orbetello	Bad	Poor to Moderate	ND	Moderate to Good	ND	Moderate to High	Moderate to High
Santa Gilla	Bad to Good	Moderate	ND	ND	ND	ND	ND

are naturally-stressed environments with a low diversity (Elliott and Quintino, 2007 and references therein), criteria to evaluate EcoQs defined in Bouchet et al. (2012) were adapted (see Table 1).

The relation between changes in environmental conditions assessed from the PERSE procedure and changes determined from benthic foraminiferal assemblages was then quantified by means of a Spearman rank correlation coefficient (Legendre and Legendre, 1998). The 95% confidence interval was estimated using a normalised bootstrap procedure with 10,000 permutations (Davison and Hinkley, 1997).

Finally, EcoQs obtained with benthic foraminifera were compared to previous studies that assessed EcoQs in the same areas using indices based on benthic macrofauna (Munari and Mistri 2007; Munari et al., 2009a; Munari et al., 2009b; Specchiulli et al., 2010; Cabiddu et al., 2014). Because we did not have access to the benthic macrofauna raw data, we were restricted to the published values (see Table 2 for details). The following indices were calculated in the aforementioned references: the Shannon index of diversity, and sensitivity-based indices BENTIX (Simboura and Zenetos, 2002), BITS (Mistri and Munari, 2008), AMBI (Borja et al., 2000), FINE (Mistri et al., 2008) and BOPA (Dauvin and Ruellet, 2007). The latest type of indices is based on the classification of species (or groups of species) into several ecological groups representing specific sensitivity levels to an increasing gradient of organic matter. Sampling for benthic macrofauna was done in 2010 in Santa Gilla, 2005 in Venice, 2003 in Orbetello, 2007 in Lesina and Varano (Munari and Mistri, 2007; Munari et al., 2009a,b; Specchiulli et al., 2010; Cabiddu et al., 2014). Although a few years passed between the sampling of benthic foraminifera and benthic macrofauna, we assumed that no changes have taken places in the environmental conditions of the study sites. Furthermore, benthic faunal patterns in Italian TWs are known to be persistent over periods of few years (Lardicci et al., 2001; Mistri, 2002; Marzano et al., 2003; Albani et al., 2007).

3. Results

3.1. Changes in environmental conditions in relation to relative reference states

Following the methodology described in Goberville et al. (2011a,b), the PERSE procedure was applied to determine *(i)* relative reference conditions (Fig. 2, right panels) inferred from the criteria defined by Bakke et al. (2010) and *(ii)* probability that each observation (*i.e.* station) belongs to the relative reference state (Fig. 2, left panels). We recall here that the probability is a measure of the distance between an observation (*i.e.* the combination of Pb, Cu, Cd, Zn and TOC concentrations) and the centroïde of the relative reference state (Fig. 2, right panels) and represents an index of environmental conditions: a probability close to 0 indicates a perturbation where the observation lies outside the relative reference state whereas a value close to 1 reflects good environmental conditions (Fig. 2, left panels).

On a site basis, the highest probabilities were reached at the Varano Lake (Fig. 2e) with two values greater than 0.85 at stations V3 and V7 and, to a lesser extent, at the Santa Gilla lagoon (Fig. 2a) with a peak at 0.71 at station SG6. These two sites were the least disturbed with environmental conditions close to the “Good” EcoQ as defined by Bakke et al. (2010). For the three other sites (Orbetello, Lesina and Venice; Fig. 2b,c and d), a reliable perturbation in their environmental conditions was detected. Probabilities that observations belong to the relative reference state remained low, suggesting that lead, copper, cadmium, zinc and/or TOC concentrations were far from the criteria defined by Bakke et al. (2010).

3.2. Ecological Quality Statuses based on benthic foraminifera

The diversity index Exp(H'$_{bc}$) was low in most of the sampling stations: < 3 for all stations sampled in Orbetello and Lesina, < 6 in most of the Santa Gilla and Venice stations and < 9 in stations situated in the Varano Lake (Table 3 and Fig. 3). Only two stations with values above 7 (*i.e.* the threshold between a “Bad” and a “Moderate” EcoQ) were determined at Santa Gilla (station SG2) and Venice (station V2), while a large amount of stations showed values above this limit in the Varano Lake (Table 3 and Fig. 3). Values of the index of environmental conditions were significantly correlated to diversity values of benthic foraminiferal assemblages (Spearman correlation = 0.53 ± 0.1; Fig. 4). Changes in diversity can therefore be related to modifications in environmental conditions.

By assessing EcoQs of Italian TWs on the basis of benthic foraminifera, we revealed that the Orbetello and Lesina lagoons were highly disturbed areas, all the stations being characterised as “Bad” EcoQ (Fig. 3c and d). At the Santa Gilla lagoon (Fig. 3a), only the station SG2 showed a good EcoQ and the majority of stations exhibited either “Poor” (such as SG1 and SG3) or “Bad” (e.g. SG5, SG11; SG17) EcoQ. This result suggests that the Santa Gilla lagoon was exposed to important environmental perturbations. No “Good” EcoQ was detected at the Venice lagoon (Fig. 3b), the stations being characterised as “Moderate” (VE2 and VE6), “Poor” (such as VE5, VE10 and VE13) or “Bad” (VE1, VE8 and VE9) EcoQs. At the Varano Lake (Fig. 3e), only the station V7 was ranked as “Good” while all the other stations ranged from “Poor” to “Moderate” EcoQs, revealing no intense perturbation in comparison to the four other study sites. Note that because the benthic foraminiferal assemblages used in this study have already been described and discussed in previous publications (Coccioni et al., 2009; Frontalini et al., 2009; Frontalini et al., 2010; Frontalini et al., 2013), we refer the reader to this literature for a site-by-site description of changes in species assemblages.

3.3. Comparison between EcoQs based on benthic foraminifera vs. benthic macrofauna

In the Varano Lake, a good agreement between EcoQs calculated from benthic foraminifera and EcoQs based on benthic macrofauna was detected with the Shannon diversity (H') (Table 2). In the Lesina lagoon, “Bad” EcoQs obtained from benthic foraminifera were similar to those based on Shannon diversity but a discrepancy was observed when the sensitivity-based BENTIX, BITS, AMBI, FINE and BOPA indices, that

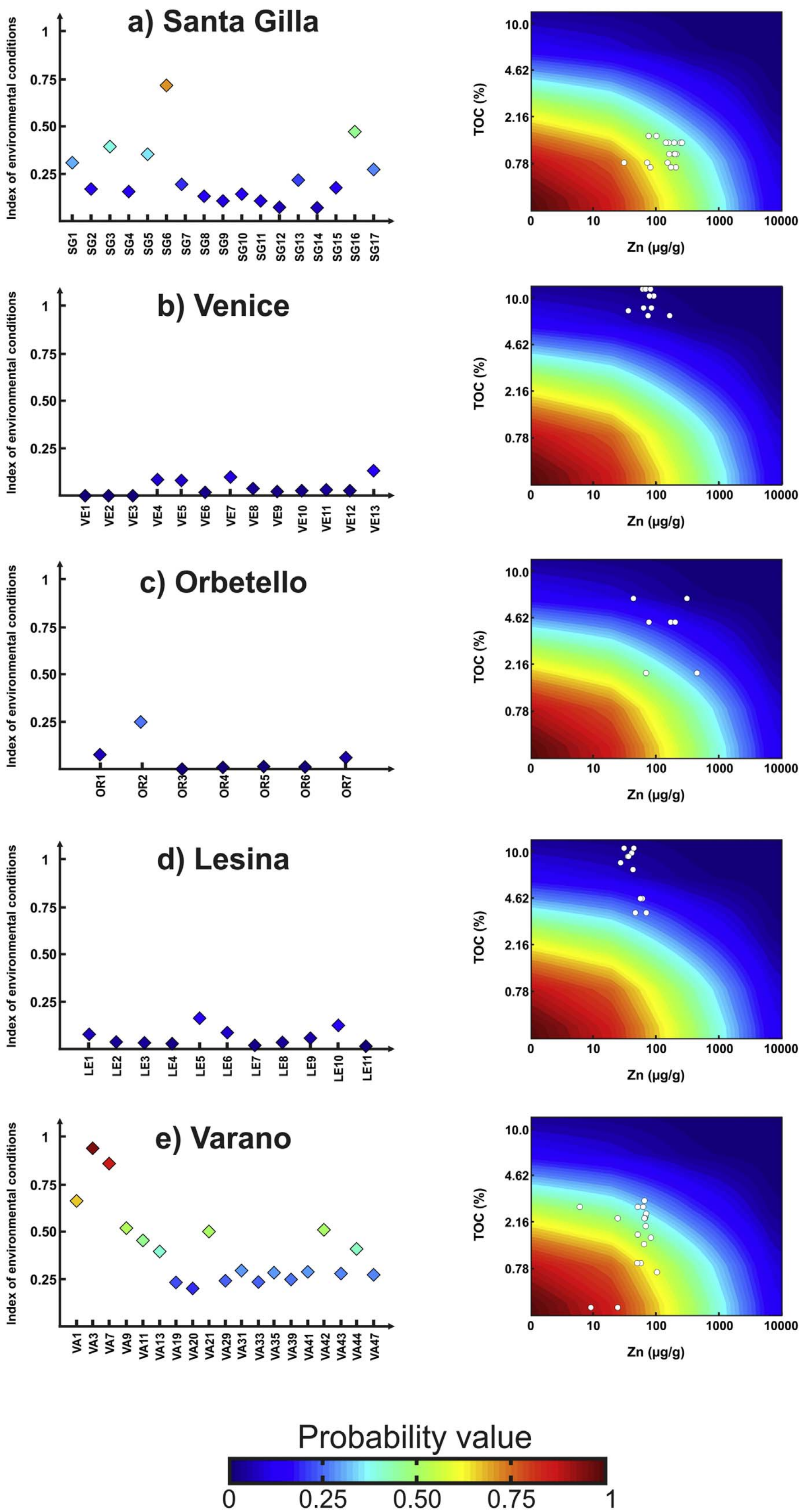

Fig. 2. Representation of the relative reference states (PERSE procedure) and probability that an observation belongs to the relative reference state of each station for a given site: a) Santa Gilla, b) Venice, c) Orbetello, d) Lesina and e) Varano. The position of the relative reference states was represented in an Euclidean space of two dimensions (right panels), the first dimension (x) being represented by the Zinc (Zn) concentration and the second dimension (y) being represented by the Total Organic Carbon content (TOC in %). Probability that an observation belongs to the relative reference state for each site was calculated and superimposed on the Euclidean space for a given station. For each site, probability values (index of environmental conditions) as function of stations were then represented (left panels).

ranked the stations from "Bad" to "High" EcoQs (Table 2), were considered. In Venice, EcoQs based on benthic foraminifera were in agreement with those determined with benthic macrofauna using H' and BENTIX, but differences appeared with the BOPA, AMBI, BITS and FINE indices (Table 2). These latter ranked some stations up to "Good" and "High" while only a "Moderate" EcoQ was obtained when benthic foraminifera were considered. In the Orbetello lagoon, benthic foraminifera (Exp(H'$_{bc}$)) and benthic macrofauna (H') converged in the

Table 3
Lead (Pb), Copper (Cu), Cadmium (Cd) and Zinc (Zn) concentrations (μg/g), Total Organic Carbon content (TOC in %) and diversity Exp(H'_{bc}) for each sampling station of the five Italian transitional waters sites.

Site	Station	Pb (μg/g)	Cu (μg/g)	Cd (μg/g)	Zn (μg/g)	TOC (%)	Exp(H'_{bc})
Orbetello	OB1	39.8	11.8	0.2	43.1	7.1	1.64
	OB2	89.4	23.1	0.2	68.3	2.8	1.94
	OB3	149.6	82.8	1.2	305.2	7.1	2.11
	OB4	244.6	108.9	1.8	441.3	2.8	1.96
	OB5	97.5	50.2	1.2	168.2	5.3	2.07
	OB6	111.1	58.1	1.1	198.7	5.3	2.23
	OB7	67.4	22.9	1.0	76.1	5.3	1.94
Lesina	LE1	33.4	35.1	0.2	59.5	5.6	1.05
	LE2	38.4	28.2	0.2	42.2	8.0	1.69
	LE3	33.5	23.4	0.2	34.8	9.4	1.57
	LE4	36.4	21.8	0.8	40.2	9.8	1.40
	LE5	29.9	31.8	0.3	45.9	4.7	1.90
	LE6	32.1	31.2	0.3	55.2	5.6	2.78
	LE7	32.7	27.4	0.2	43.5	10.4	1.97
	LE8	34.0	20.9	0.5	35.8	9.4	1.38
	LE9	29.4	17.5	0.5	26.8	8.7	2.72
	LE10	30.9	33.3	0.7	68.6	4.7	1.14
	LE11	29.7	43.5	0.2	30.4	10.4	1.37
Venice	VE1	53.6	58.8	0.5	344.7	15.1	2.71
	VE2	39.6	48.4	2.3	463.7	15.1	7.57
	VE3	29.3	34.9	0.9	200.3	15.1	4.54
	VE4	12.2	13.0	0.9	72.1	8.0	4.76
	VE5	8.9	11.3	0.2	81.4	8.8	4.15
	VE6	27.0	31.8	1.0	158.2	8.0	6.60
	VE7	9.6	7.5	0.6	61.0	8.8	–
	VE8	10.5	8.0	0.3	59.1	11.1	2.93
	VE9	15.7	15.9	0.2	65.7	11.1	2.40
	VE10	13.7	22.4	0.2	88.7	10.2	3.13
	VE11	17.8	15.8	0.2	75.6	10.2	3.91
	VE12	14.9	10.9	0.3	78.7	11.1	3.46
	VE13	7.3	8.9	0.2	35.0	8.5	4.09
Varano	VA1	2	3	0.5	6	3.8	5.44
	VA3	5	3	0.5	9	1.1	7.01
	VA7	4	11	0.5	24	1.1	10.50
	VA9	19	17	0.7	49	1.9	5.30
	VA11	24	22	0.7	56	1.9	5.81
	VA13	19	20	0.8	50	2.7	4.02
	VA19	30	22	1.0	67	3.4	4.34
	VA20	26	23	0.8	64	4.1	5.83
	VA21	21	18	0.7	49	1.9	4.31
	VA29	29	21	0.9	68	3.5	7.02
	VA31	32	25	0.8	81	2.6	4.65
	VA33	27	21	0.8	61	3.8	6.64
	VA35	30	21	0.9	67	3.0	5.14
	VA39	29	21	1.0	65	3.3	7.70
	VA41	35	33	1.2	101	1.7	7.01
	VA42	13	11	0.5	24	3.3	7.87
	VA43	29	19	0.8	64	3.3	4.90
	VA44	24	18	0.7	63	2.4	4.21
	VA47	24	17	0.8	50	3.8	8.24
Santa Gilla	SG1	56	17	0.5	101	2.5	4.91
	SG2	109	27	0.8	159	2.3	9.90
	SG3	42	14	0.4	76	2.5	4.16
	SG4	125	40	0.9	206	1.7	4.45
	SG5	110	15	0.5	81	1.7	2.95
	SG6	26	6	0.2	31	1.8	5.49
	SG7	120	30	0.9	172	1.7	2.97
	SG8	138	39	0.9	195	2.0	2.51
	SG9	164	39	1.1	210	2.0	3.08
	SG10	192	24	1.0	162	2.0	2.20
	SG11	205	26	1.3	199	2.0	1.77
	SG12	149	44	1.3	243	2.3	2.43
	SG13	80	12	1.1	194	2.3	3.25
	SG14	156	45	1.2	257	2.3	2.15
	SG15	101	27	0.9	143	2.3	2.93
	SG16	49	13	0.4	72	1.8	2.52
	SG17	59	32	0.6	152	1.8	1.75

ranking of stations in “Bad” to “Moderate” EcoQs (Table 2), but a discrepancy was observed when AMBI, FINE and BOPA indices were considered, ranking the stations from “Moderate” to “High” EcoQs (Table 2). In Santa Gilla, the best EcoQ obtained from benthic foraminifera was assessed as “Good” while it was “Moderate” when the Shannon diversity H’ was estimated on benthic macrofauna (Table 2).

4. Discussion

4.1. EcoQs assessment in Italian TWs using benthic foraminifera

The use of the Exp(H'_{bc}) index based on benthic foraminifera shows good performance in detecting both biological and environmental perturbations in the five Italian ecosystems that are classified as “Bad” to “Poor” EcoQs. Note that this is a great achievement considering the well-known difficulty in assessing EcoQs in TWs (Bouchet and Sauriau, 2008; Munari and Mistri, 2008; Lavesque et al., 2009). Our approach confirms the strong impact of human-induced forcing on Italian TWs: (*i*) aquaculture activities in Venice, Orbetello, Lesina and Varano (Marchini et al., 2008; Coccioni et al., 2009; Frontalini et al., 2010; Frontalini et al., 2013), (*ii*) industries in Venice and Santa Gilla (Degetto et al., 1997; Coccioni et al., 2009; Frontalini et al., 2009), (*iii*) agricultural effluents in Venice, Orbetello, Lesina and Varano (Lardicci et al., 2001; Munari and Mistri, 2008; Frontalini et al., 2010; Borja et al., 2011), or (*iv*) urban discharges in Venice, Orbetello and Lesina (Lardicci et al., 2001; Munari and Mistri, 2008; Borja et al., 2011). Such results are consistent with previous studies in the same areas that have reported an increase in tolerant species associated with a decrease in diversity, leading to degraded foraminiferal communities (Albani et al., 2007; Coccioni et al., 2009; Frontalini et al., 2009; Frontalini et al., 2010; Frontalini et al., 2013). However, these conclusions have been reached using complex multivariate analyses, that are more difficult to interpret than the Exp(H'_{bc}) index in terms of environmental monitoring.

Our results, in line with previous studies performed on benthic foraminifera in Norwegian fjords (Bouchet et al., 2012), in the harbour of Boulogne-sur-Mer (France) (Francescangeli et al., 2016) and in the Oslofjord (Dolven et al., 2013), support that the Exp(H'_{bc}) index based on benthic foraminifera proposed by Bouchet et al. (2012) allows a straightforward assessment of the EcoQ of water bodies. Here, we clearly disprove Barras et al. (2014) who reported that diversity indices are not an appropriate tool to assess the EcoQ in the Mediterranean Sea. Although designed in Norwegian fjords, the foraminiferal method can therefore be used in different types of environments and not only in Norway. Originally, criteria were developed against a decreasing gradient of bottom-water dissolved oxygen concentrations (Bouchet et al., 2012). In this study, the applicability of the foraminiferal method in the context of organic matter and trace elements pollutions is shown. The Exp(H'_{bc}) index allows a synthetic and easily interpretable representation of the health of foraminiferal communities and by extension a rapid assessment of EcoQs. From an operational point of view, this index is easy to apply by managers and stakeholders, making this method an efficient and valuable alternative to sophisticated statistical procedures.

4.2. Benthic foraminifera to complement benthic macrofauna to assess EcoQs

The characterisation of EcoQs using diversity indices based on either benthic foraminifera or benthic macrofauna leads to similar conclusions. In Italian TWs, changes in both foraminifera and macrofauna diversity along an environmental gradient are thus comparable. Similarly, changes in EcoQs of the Oslofjord characterised by the Exp (H'_{bc}) index calculated on either foraminifera or benthic macrofauna were congruent and highly related to environmental changes induced by pollution disturbances (Dolven et al., 2013). This confirms

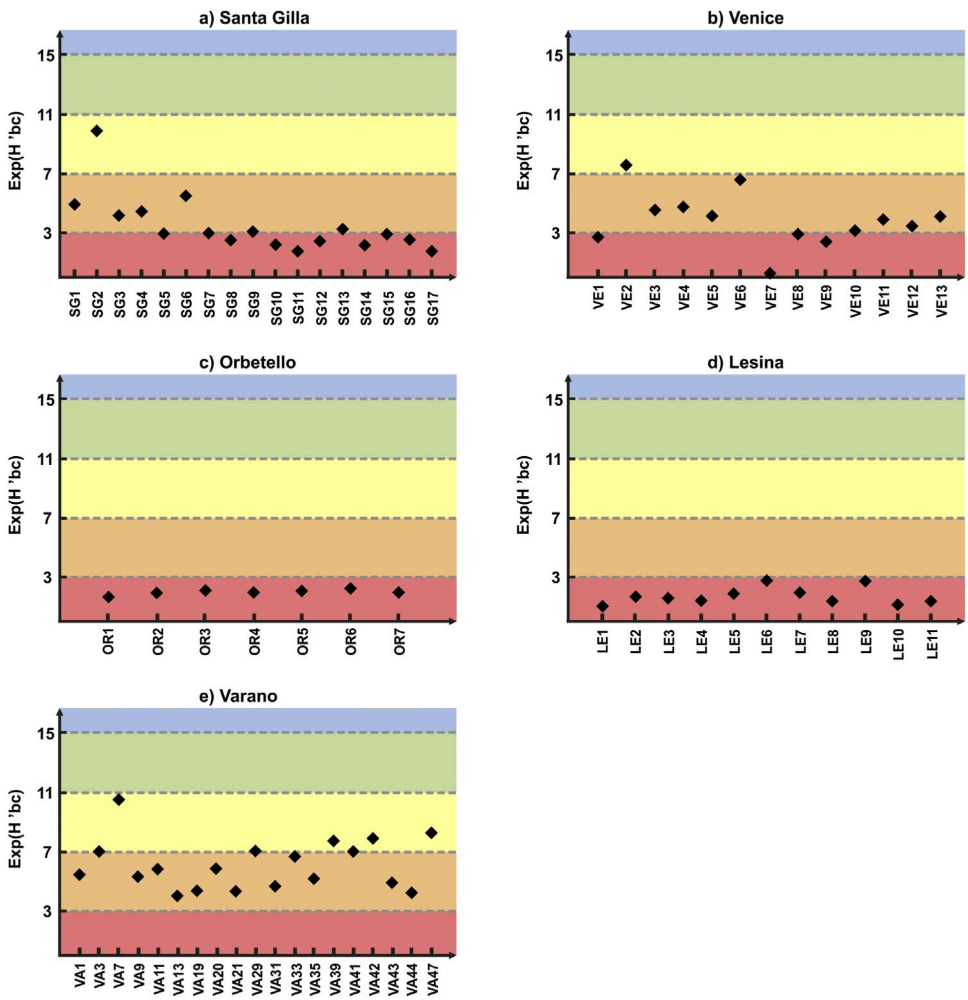

Fig. 3. Diversity index Exp(H'$_{bc}$) values and corresponding Ecological Quality status (red: poor, orange: bad, yellow: moderate, green: good and blue: excellent) for each sampling station of the five Italian transitional waters sites: a) Santa Gilla, b) Venice, c) Orbetello, d) Lesina and e) Varano.

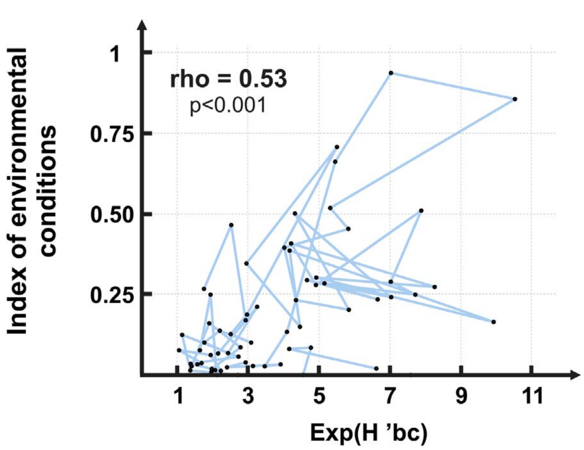

Fig. 4. Relation between changes in environmental conditions estimated from the PERSE procedure and changes in benthic foraminiferal diversity assessed by the Exp(H'$_{bc}$) index.

Wlodarska-Kowalczuk et al. (2013) who have shown that foraminiferal and macrofaunal diversity based on H' were positively correlated in an Arctic glacial fjord. A similar pattern, *i.e.* a decrease in both benthic foraminiferal and macrofaunal diversity (H'), was also observed in the Firth of Clyde (Scotland) as a result of an organic matter pollution (Mojtahid et al., 2008).

Differences occurred when comparing results obtained with foraminifera diversity-based index and macrofaunal sensitivity-based indices. Similar differences have been reported in studies comparing the efficiency of sensitivity-based indices applied on both groups. In the Firth of Clyde, while similar diversity (H') patterns were reported, patent differences in sensitivity-based indices have been observed (Mojtahid et al., 2008). In a study on the impact of oil-drill mud disposal, benthic foraminifera were more sensitive than macrofauna to environmental degradations (Denoyelle et al., 2010). Biotic indices based on benthic macrofauna are currently facing issues to reliably assess EcoQs in Italian TWs (Munari and Mistri, 2008, 2010; Borja et al., 2011; Munari and Mistri, 2012), which may explain the discrepancies between EcoQs obtained with macrofauna and those calculated from foraminifera. In contrast to the foraminiferal method, most biotic indices based on macrofauna rely on species sensitivity/tolerance according to the Pearson and Rosenberg paradigm (1978). Macroinvertebrates are classified into groups with similar levels of sensitivity/tolerance to an enrichment in organic matter in the sediment (Glémarec and Hily, 1981; Borja et al., 2000): the more sensitive species in a community, the better EcoQ, and *vice versa*. Such assumption is a clear limitation for a reliable assessment of the EcoQ of TWs with macrofaunal sensitivity-based indices (Bouchet and Sauriau, 2008; Munari and Mistri, 2008; Lavesque et al., 2009). Italian TW sediments being naturally rich in OM, tolerant and opportunistic species are promoted (Marchini et al., 2008; Munari and Mistri, 2008; Munari et al., 2009a), leading to a natural shift towards moderate to bad EcoQs (see aforementioned references). As we have shown, the diversity index Exp(H'$_{bc}$) associated with benthic foraminifera might therefore be an appropriate method for EcoQ monitoring in Italian TWs.

4.3. Implications for environmental studies

While recent studies, including ours, argue that benthic foraminifera associated with the Exp(H'$_{bc}$) index is an accurate method to evaluate the health of water bodies, another initiative, led by the FOBIMO international group (FOraminiferal BIoMOnitoring), focuses mostly on the sensitive-species approach to develop a foraminiferal biotic index (Alve et al., 2016): the Foram-AMBI index. Promising application of the Foram-AMBI index in marine North-East Atlantic and Arctic fjords, continental shelves and slopes have been recently reported (Alve et al., 2016). The concept of "indicator species" in biomonitoring studies has been, however, recently questioned for macrofauna (Spilmont, 2013; Zettler et al., 2013). Species sensitivity/tolerance along a gradient of perturbation might change depending on local environmental conditions such as salinity (Zettler et al., 2013). Macroinvertebrate species are known to be plastic enough to adapt to their environment: they could modify their autoecology requirements along environmental gradients (see review in Zettler et al., 2013 and reference therein) and the tolerance spectrum of species might be wider and more intricate than a simple categorisation, *i.e.* "sensitive species" or "tolerant species". Because the same concerns may arise from the use of benthic foraminifera, alternative approaches such as the diversity index Exp(H'$_{bc}$) have to be considered and tested.

In this study, thresholds between the different classes of EcoQs have been adapted for a reliable assessment of EcoQs using the foraminiferal method. As aforementioned, TWs, including Italian ecosystems, are naturally stressed environments with a low diversity, making unsuitable the criteria determined in Norwegian fjords where diversity is commonly higher (Bouchet et al., 2012). In that sense, benthic foraminifera are not an exception and the same applies for benthic macrofauna in other TW systems (Dauvin, 2007): BENTIX index thresholds, for instance, depend on sediment type (*i.e.* mud or sand; Simboura and Reizopoulou, 2007). In the same way as for benthic macrofauna (Bouchet and Sauriau, 2007; Blanchet et al., 2008; Lavesque et al., 2009), the foraminiferal method should be used with caution to prevent a wrong EcoQ assessment of TWs. However, local reference conditions might overcome such limitations. For instance, a more robust assessment of the EcoQ of Danish coastal waters was obtained with eelgrass using site-specific reference conditions instead of type-specific criteria (Krause-Jensen et al., 2005). The same conclusions were reached with the foraminiferal method in the Oslofjord (Norway; Dolven et al., 2013) and in the Boulogne-sur-Mer harbour (France; Francescangeli et al., 2016).

The WFD and the MSFD identify four options to define reference conditions or to set targets: *(i)* a comparison with a pristine/undisturbed area, *(ii)*using historical data, *(iii)* performing modelling approaches or *(iv)* using expert judgment (Muxika et al., 2007). The assessment of the EcoQ or the Environmental Status (ES) of a site is based on the comparison between a value, calculated from any index, and a given reference: the closer to the reference, the better the EcoQ or the ES. Setting "true" or "reliable" reference conditions/targets is therefore crucial (Borja et al., 2012), although each option has its own strengths and weaknesses (Borja et al., 2012). For example, it is a conundrum to characterise pristine areas in coastal systems as most of these regions are already under the influence of human activities (Diaz and Rosenberg, 2008). Hindcasting approaches, *i.e.* the use of historical information, imply the existence of long-term monitoring or fossil records of taxa used to characterise the EcoQ or the ES. Such an approach is problematic when macrofaunal species are considered. While molluscs and gastropods are well preserved over time (Poirier et al., 2009), this is not the case for other taxa and an important part of the biological signal contained in macrofauna assemblages is lost (Poirier et al., 2009). As a consequence, expert judgment is mostly used to define references and targets (Muxika et al., 2007; Bouchet and Sauriau, 2008), but remains subjective (Muxika et al., 2007). In contrast to benthic macrofauna, fossil remains of benthic foraminifera can provide information about long-term environmental and biological changes, their biological signal being well preserved over time (Alve et al., 2009; Dolven et al., 2013; Francescangeli et al., 2016). Although taphonomical processes should be considered in palaeoecological-studies, it should not be an issue in Italian TWs. Benthic foraminifera can therefore enable the reconstruction of palaeo-environments used as reference conditions. It would allow us to better disentangle natural- from human-induced changes, including the detection of the effects of environmental pollution (Alve, 1991; Dolven et al., 2013; Francescangeli et al., 2016; Alve et al., 2009).

5. Conclusions

Our work corroborates that benthic foraminifera are a promising tool to evaluate EcoQs in marine ecosystems. Their use still requires further investigations, however (*e.g.* other ecosystems or sources of pollution). In this study, we showed that the diversity index Exp(H'$_{bc}$), calculated on benthic living foraminifera, is a relevant metric to assess the EcoQ in highly variable ecosystems such as Italian TWs. By comparing EcoQs assessment obtained using the foraminiferal method with those calculated with macrofaunal methods, we demonstrated that benthic foraminifera may be more appropriate than benthic macrofauna to accurately evaluate the health of Italian TWs.

Changes in environmental conditions, characterised by the PERSE procedure, and changes in benthic foraminiferal assemblages, assessed from the diversity index, are significantly related, suggesting that both methods converged in defining relative reference conditions in Italian TWs. Living benthic foraminiferal species being good indicators of environmental modifications, fossilised organisms might therefore be considered as appropriate candidates to define local and/or regional reference conditions. This approach, more objective than methods such as "expert judgment", will allow to better monitor the EcoQ of water bodies with the aim to improve management and conservation plans.

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