

Animal-sediment relationships: Evaluating the ‘Pearson-Rosenberg paradigm’ in Mediterranean coastal lagoons

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Abstract

In the present study, we investigated the applicability of the Pearson-Rosenberg (P-R) conceptual
25 model describing a generalized pattern of response of benthic communities in relation to organic
enrichment to a specific typology of marine systems, i.e. coastal lagoons. The study was
performed with independent matching data on the structure of macrobenthic communities and
total organic carbon (TOC) content of sediments obtained from 349 stations representing three of
the most ecologically and economically relevant coastal lagoons in the Mediterranean Sea, i.e. the
30 lagoons of Cabras, Orbetello, and Venice (Italy). Consistent with P-R model predictions, we
found two different peaks in benthic diversity and abundance at low ($> 2.5-5 \text{ mg g}^{-1}$) and high ($> 25-30 \text{ mg g}^{-1}$) TOC ranges, respectively. We identified TOC thresholds indicating that risks of
reduced benthic diversity from organic loading and other associated stressors in sediments should
be relatively low at TOC values $<$ about 10 mg g^{-1} , high at TOC values $>$ about 28 mg g^{-1} , and
35 intermediate at values in-between. Predictive ability within these ranges was high based on
results of re-sampling simulation. Our results validate and support a prior independent study that
identified similar TOC thresholds for assessing risks of benthic impacts in samples from seven
coastal regions of the world. While not a measure of causality, it is anticipated that these TOC
thresholds should serve as a general screening-level indicator for evaluating the likelihood of
40 reduced sediment quality and associated bioeffects in such eutrophic systems of the
Mediterranean Sea, which also are exposed to a variety of stressors from multiple human uses.

Keywords: Macrobenthos; Total organic carbon (TOC); Benthic-TOC relationships; Ecological
indicators; Pollution impacts; Eutrophication; Benthic diversity; Coastal lagoons; Mediterranean
45 Sea

1. Introduction

Coastal marine ecosystems are increasingly affected by environmental stress and degradation due to pollution and other anthropogenic factors. A large number of research programmes worldwide have addressed these problems within various coastal regions and have produced highly useful comprehensive datasets on environmental and biotic conditions within each system. A growing number of new tools, methods, and models for assessing the health of these systems have emerged as well, along with the recognition that a wide suite of approaches would be best for such purposes rather than any one single indicator (Magni et al., 2005a; Dauvin, 2007; Borja and Dauer, 2008). Furthermore, it has been recognized that there would be a tremendous advantage in bringing such information and resources together through collaborative efforts in order to provide consistent and comprehensive sets of indicators and related data for future global comparisons (Costello and Vanden Berghe, 2006). At the same time, while consistent and globally-applicable approaches are important, there also is need for adopting monitoring and analytical approaches that recognize and account for natural variations among various regions and unique properties of specific systems (e.g. oligotrophic vs. eutrophic systems, coastal sites vs. estuaries). The final aim is to develop recommendations and indicators that hopefully will provide useful guidance for future coastal research and management applications.

The use of ecological indicators is central to such an approach. While there are many qualities of a good indicator (e.g., see reviews by Cairns et al., 1993; Fisher et al., 2001; European Environment Agency, 2005; Magni et al., 2005a; Rees et al., 2006; UNESCO, 2006; ICES, 2008), a particularly important feature is the presence of a strong stressor-response relationship with quantifiable thresholds to allow one to convey the current status of condition relative to some optimal environmental quality target either to maintain (if the system is already in a healthy state) or to achieve (if the system is disturbed and in need of restoration). As an example, abiotic environmental attributes, such as measures of organic enrichment or chemical contamination and toxicity in sediments, might be the stressor component while measures of key biological

attributes, such as benthic species richness, abundance, or biomass would represent response variables. A classic example of such a stressor-response relationship is provided in the graphical model by Pearson & Rosenberg (P-R, 1978), describing a generalized pattern of benthic community response along a gradient of organic enrichment. Hyland et al. (2005) recently expanded upon the P-R model by using it as a conceptual basis for defining lower and upper thresholds in total organic carbon (TOC) concentrations corresponding to low vs. high levels of benthic species richness in samples from seven coastal regions of the world. Specifically, it was shown that risks of reduced macrobenthic species richness from organic loading and other associated stressors in sediments should be relatively low at TOC values < about 10 mg g⁻¹, high at values > about 35 mg g⁻¹, and intermediate at values in-between. While not a measure of causality (i.e. to imply that the observed bioeffect was caused by TOC itself), it was anticipated that these TOC thresholds may serve as a general screening-level indicator, or symptom, of ecological stress in the benthos from related factors. Such factors may include high levels of ammonia and sulfide or low levels of dissolved oxygen associated with the decomposition of organic matter, or the presence of chemical contaminants co-varying with TOC in relation to a common controlling factor such as sediment particle size.

In the present study, we aimed to assess the applicability of benthic-TOC relationships and associated thresholds for one particular typology of coastal systems, i.e. Mediterranean coastal lagoons. Our choice was based on the hypothesis that these relationships might be very different or invalid in the case of such eutrophic, organic-enriched systems as are many of the coastal lagoons in the Mediterranean Sea. To this end, matching data on the structure of macrobenthic communities and TOC content of sediments were obtained and analyzed, through the collaborative efforts of several institutions and scientists, from 349 stations representing the three Mediterranean Sea coastal lagoons of Cabras, Orbetello, and Venice (Italy).

2. Materials and methods

2.1 Lagoons investigated

The lagoons of Cabras, Orbetello and Venice are shallow, eutrophic bodies of water geographically belonging to three different marine sub-basins in which the Mediterranean Sea is usually subdivided (UNEP/FAO/WHO/IAEA, 1990; Magni et al., 2008a): the Southwestern Mediterranean Sea, the Tyrrhenian Sea, and the Adriatic Sea, respectively (Fig. 1). They are characterized by nano- (Cabras and Orbetello lagoons) or micro-tidal (Venice lagoon) tides (Tagliapietra and Volpi Ghirardini, 2006). According to the Koeppen-Geiger-Pohl Climatic Classification (e.g. Peel et al., 2007), the Cabras and Orbetello lagoons have a Csa climate (i.e. Mediterranean Mild with dry, hot summer), while the Venice lagoon experiences a Cfa climate (i.e. Humid subtropical Mild with no dry season, hot summer). These lagoons represent three of the most economically and ecologically relevant, as well as widely studied, coastal lagoons in Italy and the Mediterranean Sea. Historically, they have a high economic rating due to fishery activities including both fishes (e.g. *Liza ramado*, *Mugil cephalus*, *Anguilla anguilla*, *Sparus aurata*, *Dicentrarchus labrax*) and shellfish (e.g. *Mytilus galloprovincialis*, *Ruditapes philippinarum*). However, like many other Mediterranean coastal lagoons, they also tend to be subjected, especially during summer and periods of climatic stasis, to dystrophic events causing massive mortalities of benthic macroinvertebrates and fishes. Eutrophication and organic over-enrichment of sediments, resulting in the subsequent release from sediments of toxic hydrogen sulphide, are thought to be major causes of these events (Lardicci et al., 1997; Tagliapietra et al., 1998; Magni et al., 2005b, 2008b). Several studies have been conducted on benthic communities of the Cabras, Orbetello, and Venice lagoons (e.g. see Magni et al. 2004, 2005b, 2008a; Como et al., 2007, for the Cabras lagoon; Lardicci et al., 1993, 1997, 2001; Lardicci and Rossi 1998, for the Orbetello lagoon; Tagliapietra et al., 1998, 2000; Pessa, 2005, for the Venice lagoon) and the reader is referred to these references for more detailed accounts of the individual systems.

2.2 Dataset

Matching data on the structure of macrobenthic communities and organic carbon content of
125 sediments were obtained from 59, 108, and 182 stations from multiple sampling efforts in the
lagoons of Cabras (De Falco et al., 2004; Magni et al. 2004, 2005b), Orbetello (Lardicci et al.,
1997, 2001) and Venice (Tagliapietra et al., 2000; Frangipane, 2005; Pessa, 2005) respectively,
resulting in a total of 349 stations (Table 1). There were some differences in methods used to
generate the data among the various studies (e.g., variations in sieve sizes, surface area of
130 sampling gear, method of organic matter determination). However steps were included in the
present analysis to account for such differences. For example, synoptic data of total organic
carbon (TOC) measured by a CHN analyzer and organic matter (OM) measured by loss on
ignition (LOI) were available for a subset of stations in each lagoon (e.g. Frangipane et al., in
press; Magni et al., 2008a). Where organic carbon was measured only as OM content by LOI,
135 site-specific equations were used to convert to TOC, taking into account the analytical conditions
(i.e. temperature and time of ignition) used in each study for LOI (see also Frangipane 2005;
Magni et al., 2008a). These equations were as follows: Cabras lagoon $TOC = 0.30 LOI (500^{\circ}C \times 3 h)$ ($R^2 = 0.75, P < 0.001$), Orbetello lagoon $TOC = 0.27 LOI (450^{\circ}C \times 4 h) - 0.28$ ($R^2 = 0.85, P < 0.001$), and Venice lagoon $TOC = 0.48 LOI (350^{\circ}C \times 16 h) + 0.1$ ($R^2 = 0.96, P < 0.001$). Figure
140 2 shows the ranges of TOC content of sediments of the three lagoons after standardization.
Salinity values were also available for most stations (333 out of 349). Their ranges are also given
for each lagoon in Fig. 2.

As for macrobenthos, numbers of individuals of each benthic species (or lowest practical
taxon) in a sample were recorded by station and lagoon. Taxonomy consistency across different
145 studies was carefully checked. Taxa were in most cases identified with similar experience levels,
e.g. by similarly trained staff from the same institution in the case of the Cabras and Orbetello
lagoons. Furthermore, when differences in the level of taxonomic resolution were observed in the

raw data, the higher taxonomic level was used consistently across lagoons. This need occurred for the syllid polychaetes which were classified to the species level in the Orbetello and the Cabras lagoons (17 and one species found, respectively) and to the family level in the Venice lagoon. In this case, all Syllids in a given sample were grouped as one family. Nevertheless, a comparative analysis of various diversity measures [e.g. H' and $E(S_{10})$] calculated for the Orbetello samples using Syllids as individual species and merged to family showed no significant differences, with H' and $E(S_{10})$ averaging $99\% \pm 2$ and $98\% \pm 3$, respectively. This is consistent with the low contribution of Syllids to the total number of taxa in our dataset and their overall limited occurrence in lagoonal environments as compared to coastal waters (Mistri and Munari, 2008). Total abundance was also corrected for sample-size differences by standardizing the data to a per- m^2 basis (i.e. density). Additional methods to account for potential sample-size variations (resulting from different grab and sieve sizes) were used in the data analysis step as well (see below).

2.3 Data Analysis

Three benthic variables were selected to examine in relation to the TOC data: total macrobenthic density (number of individuals m^{-2} for all species combined), and two diversity measures, Shannon index, H' , calculated with base-2 logarithms (Shannon and Weaver, 1949) and Hurlbert's $E(S_n)$ (Hurlbert, 1971). $E(S_n)$ is the expected number of species present in an increasingly rarefied sample of n individuals randomly selected (without replacement) from a finite collection of N individuals and S species. With this measure, meaningful comparisons of species richness among collections of different sizes can be made by adjusting the collections to a common size (n). Such a feature was particularly suitable for the present study in which we sought to examine benthic diversity in relation to TOC among samples collected with varying types of sampling gear. While $E(S_n)$ is generally unaffected by sample-size variability, it is not sample-size independent when $N < n$. Nevertheless, a low value of n ($n = 10$) was chosen in order

that low-abundance samples could be included in the analysis. H' also was used as an example of
175 a highly sample-size dependent diversity measure to evaluate possible deviations in the patterns
generated by different indices along a gradient of organic enrichment.

The analysis of the merged dataset was conducted following the approach used by Hyland et
al. (2005). Simple X - Y plots of macrobenthic density, richness, and diversity versus TOC content
were generated as a tool for examining basic patterns in the data and comparing them against the
180 conceptual model. TOC content (mg g^{-1} , plotted on the X axis) was divided initially into 10
discrete intervals as follows: ≤ 2.5 , $> 2.5-5$, $> 5-10$, $> 10-15$, $> 15-20$, $> 20-25$, $> 25-30$, $> 30-35$,
 $> 35-40$, and > 40 . Mean density and diversity measures among stations within a specific TOC
interval, and the 95% confidence intervals, were plotted across each of the TOC intervals. The
resulting curves were examined to look for their consistency with the P-R model and main
185 breakpoints in the data.

Two quantitative methods were used to determine the location of TOC thresholds associated
with the largest changes in benthic measures. These were identified as a lower TOC critical point
at which diversity starts to decline and an upper TOC critical point at which the decline starts to
level out. In the first approach, we used ANOVA as an exploratory tool to identify TOC
190 thresholds that maximized differences in diversity among the three TOC ranges resulting from
various combinations of upper and lower TOC values. Upper and lower TOC thresholds were
derived by selecting the two values that produced the highest F -statistic (Sokal and Rohlf, 1981).
In the second approach, a standard sigmoid dose-response curve was fitted to the data using non-
linear least-squares regression (Bates and Watts, 1988). The function was of the form:

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$$f(x) = a_0 + a_1 / (1 + e^{a_2 + a_3 x}) \quad [1]$$

where $x = \text{TOC (mg g}^{-1}\text{)}$ and a_0 , a_1 , a_2 , and a_3 are parameters selected by the regression procedure
to minimize the sum of squared deviations from the fitted curve. Upper and lower TOC thresholds
were calculated by determining minima and maxima of the second derivatives.

The two TOC thresholds determined from each analysis were used to separate samples into
200 three groups of low, medium, and high levels of TOC. To evaluate the predictive ability of these
thresholds as indicators of change in benthic diversity, a re-sampling simulation (Lunneborg,
1999) was used to estimate the probability of observing reduced diversity with increasing TOC
across these three ranges. Simulations consisted of 10 iterations of 250 pair-wise comparisons of
randomly selected samples (with replacement) from each group. Probabilities of reduced diversity
205 were computed for the following combinations: $E(S_{10})$ or H' in Low TOC range $>$ $E(S_{10})$ or H' in
Medium TOC range; $E(S_{10})$ or H' in Low TOC range $>$ $E(S_{10})$ or H' in High TOC range; $E(S_{10})$ or
 H' in Medium TOC range $>$ $E(S_{10})$ or H' in High TOC range. These probabilities, which can range
from 0.5 to 1.0, are a measure of the power of a threshold in separating samples with higher and
lower diversity. Probabilities closer to 1.0 are indicative of thresholds with higher discriminatory
210 power, while probabilities close to 0.5 indicate that TOC alone has no explanatory power with
respect to reductions in diversity. Type I error probabilities were computed as a basis for testing
the null hypothesis that when comparing samples taken from two groups, the probability of
observing reduced diversity in the higher TOC range is equal to 0.5 based on one-sided t -tests.

All statistical tests were conducted using either SAS (SAS Institute, USA) or S-Plus (Math
215 Soft, Inc., USA).

3. Results

3.1 Density and diversity patterns in relation to TOC

Patterns of benthic density and diversity in relation to increasing TOC content are illustrated
220 in Fig. 3. Density largely fluctuated at low TOC ($< 5 \text{ mg g}^{-1}$) and showed a moderate increase
over a wide TOC range, with a peak at 25-30 mg g^{-1} , followed by a decline (Fig. 3a). In contrast,
the two diversity measures peaked at TOC between about 2.5-5 mg g^{-1} , began declining between
5-10 mg g^{-1} , and then reached a minimum around 35-40 mg g^{-1} (Fig. 3b, c).

225 3.2 TOC thresholds for assessing reductions in diversity

As described in Section 2.3, two different quantitative methods were used to help pinpoint the lower and upper TOC thresholds: selecting the two values that produced the highest F -statistic from a series of ANOVAs performed on various combinations of upper and lower TOC values (Method 1); and calculating inflection points of a sigmoid dose-response function fitted to the original data (Method 2). A plot of the F -values (Method 1) and the fitted curves (Method 2) are shown in Figs. 4 and 5, respectively.

The two methods produced very similar TOC thresholds for both diversity measures, each with a highly significant F -statistic. The main exception was the low TOC threshold for H' using Method 1 (maximum F -statistic), which differed the most from the others (Table 2). In particular, lower and upper thresholds produced by Method 1 were 11 and 29 mg g⁻¹ for $E(S_{10})$, and 15 and 30 mg g⁻¹ for H' , respectively. This discrepancy could be related to a lower suitability of H' , as a highly sample-size dependent diversity measure, for analyzing our merged dataset. Lower and upper thresholds produced by Method 2 (dose-response function) for $E(S_{10})$ and H' were identical, i.e. 10 and 28 mg g⁻¹. Given these patterns, TOC concentrations of 10 and 28 mg g⁻¹ were selected as lower and upper thresholds for further analysis. Thus, the likelihood of observing a decline in benthic diversity in relation to increasing TOC is expected to be relatively low at concentrations < about 10 mg g⁻¹, high at concentrations > about 28 mg g⁻¹, and intermediate at concentrations in-between. On a related note, differences in mean values of both diversity measures among the three TOC ranges (low, moderate, high), tested using ANOVA, were highly significant, with Type I error probabilities near zero, regardless of which set of thresholds (11 and 29, or 10 and 28) were used to define these ranges. This is true even after applying a Bonferroni correction to account for the multiple-comparisons issue associated with the Method 1 (maximum F -statistic) procedure.

3.3 Predictive ability of TOC thresholds

250 Results of re-sampling simulation (Table 3) indicated that there is a very high probability of
having high, medium, and low mean diversity within low, medium, and high ranges of TOC,
respectively. The values for all group comparisons in Table 3 are significantly different from a
probability of 0.5 (all $P < 0.0001$). These are the Type I error probabilities associated with
rejecting the null hypothesis that when comparing samples taken from two groups, the probability
255 of observing reduced diversity in the higher TOC range is equal to 0.5 based on one-sided t -tests.
Based on these results, it appears that there is high predictive ability across the TOC ranges
defined by these thresholds. On a related note, an analysis of stations with TOC in the low range
(i.e. $\leq 10 \text{ mg g}^{-1}$) and benthic diversity in the high range (restricted to stations with $E(S_{10})$ values
above the mean high range of the original lagoon) revealed that 78% of them (39 out of 50
260 stations) are in more “vivified” (unsecluded) areas of the lagoons (e.g. seaward –with a higher
water renewal) removed from major anthropogenic influences, and thus reflecting relatively
undisturbed conditions. In contrast, 88.5% of the stations (54 out of 61) in the high TOC range
(i.e. $> 28 \text{ mg g}^{-1}$) and with lower diversity were closer to point sources of human-induced stress
(e.g. Orbetello and Cabras stations) or in secluded areas (e.g. Venice stations, located far from the
265 sea inlets), and thus reflecting, directly or indirectly, more disturbed conditions. Whereas this
analysis was based on our personal, yet extensive, knowledge of the studied lagoons and the
location of sampling stations (i.e. “expert-judgements”), this analysis helps to illustrate that the
identified thresholds appear to be realistic and predictive of disturbed condition where clear
evidence of disturbance exists.

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4. Discussion

4.1 Animal-stressor relationships and the Pearson-Rosenberg model in Mediterranean coastal lagoons

The need to develop appropriate tools or indicators for assessing the ecological quality of
275 coastal marine and transitional waters in a regulatory framework has sparked considerable

discussion among the scientific community worldwide (e.g. Dauvin, 2007; Borja and Dauer, 2008; Rees et al., 2008). Within the European Water Framework Directive (WFD; 2000/60/EC), descriptive approaches and expert judgments are used for different biological components and reference conditions (e.g. Ballesteros et al., 2007; Muxika et al., 2007). Yet, also recognized is the importance of quantitative data for precision and accuracy, stressor-response relationships, predictive modeling, and sound ecological theory (Magni et al., 2005a; Orfanidis, 2007; Josefson et al., 2008; Lyche Solheim et al., 2008). A significant challenge in assessing stressor-response relationship in transitional waters, such as estuaries and coastal lagoons, is that they are under the influence of multiple factors and have a great internal patchiness and heterogeneity, which can often bias the application of the most common indicators and indices of environmental quality and health status (Dauvin, 2007; Elliott and Quintino, 2007; Ruellet and Dauvin 2007). An example of such a complexity and uncertainty in ecological-quality assessment is provided by Mediterranean coastal lagoons. These usually are shallow, highly productive ecosystems where benthic components and processes play an important regulatory function for the whole system (Viaroli et al., 2004; Marinov et al., 2007). Yet, most common benthic indices have shown limits in defining their ecological status (Munari and Mistri, 2008). Under these circumstances, one should identify a set of basic benthic/sedimentary variables or ecological attributes indicative of operative ecosystem properties and functions that could be integrated and used for classification and quality-assessment purposes (e.g. Viaroli et al., 2004; Magni et al., 2008a).

The P-R model (1978) has been widely used over the past three decades to describe distributional and spatial changes of marine benthic communities along a gradient of organic enrichment. This conceptual model can be associated with a stressor-response relationship where the organic enrichment of sediments is the stressor component and the measures of macrobenthic attributes, i.e. number of species, abundance and biomass, are the response variable. In this study, we evaluated the applicability of the P-R model and the robustness of TOC thresholds for assessing the likelihood of benthic community impairment in Mediterranean coastal lagoons, as

generally eutrophic ecosystems. Our results indicate that abundance and diversity patterns in lagoon ecosystems (Fig. 3) are consistent with the P-R model predictions. For example, there may be a co-existence of species with varying life-history strategies and levels of tolerance to stress throughout the intermediate TOC range. As TOC increases within this range, heartier opportunistic species are able to maintain high abundances even though other more sensitive species may be dropping off. In the present study, this is indicated by the highest abundances at a high (> 25-30 mg g⁻¹) TOC range, where benthic diversity had markedly declined (Fig. 3). Yet, abundance does not appear to be a good variable to use for deriving both lower- and upper-range TOC thresholds, because there is no lower-end inflection point at which the curve begins to show a gradual decline. In contrast, diversity measures show a maximum in the low TOC range, a gradual decline over the intermediate TOC range, and a minimum in the high TOC range (Fig. 3b, c). This pattern was similar to that described by Hyland et al. (2005) and suitable for identifying ranges in TOC that could be used to assess low, moderate, and high risks of an impaired benthos (i.e. reduced diversity) along a gradient of organic enrichment in Mediterranean coastal lagoons.

4.2 Organic enrichment, TOC thresholds and data uncertainty

It is recognized that Mediterranean coastal lagoons generally are organically enriched systems, increasingly receiving sources of anthropogenic disturbance from multiple human uses (Como et al., 2007; Magni et al., 2008a; Munari and Mistri, 2008). Yet, the term “organic enrichment” is often used in a relative or conceptual manner, while there is an increasing need for a quantitative and broadly applicable classification of organic enrichment in marine sediments in order to assess changes in the benthos (Hargrave et al., 2008). This study now provides a quantitative framework for evaluating the biological significance of “organic enrichment” in Mediterranean coastal lagoons, as related to an increasing risk of benthic impact. In particular, our results showed that the probability of having a reduced diversity from organic loading and other associated stressors in sediments should be relatively low in the low TOC range (<10 mg g⁻¹) and

high in the upper TOC range ($>28 \text{ mg g}^{-1}$), being the latter threshold indicative of excessive organic enrichment. For the same reason, TOC values within the intermediate group ($>10\text{-}28 \text{ mg g}^{-1}$) should comprise stations with moderate to high organic enrichment, which in some cases may also have a naturally low benthic diversity (Fig. 5), as is typical of lagoon ecosystems. The largest variation of benthic diversity (Fig. 5) and the highest numbers of individuals (Fig. 3a) found within the intermediate group consistently suggest the occurrence here of fewer tolerant and opportunistic species.

Predictive ability within these TOC ranges was high based on results of re-sampling simulation (Table 3), suggesting that these particular breakpoints in the data provide a reasonable framework for assessing effect of organic enrichment on lagoonal benthic diversity. We acknowledge that predictive ability of TOC thresholds is not free of uncertainty. We still found much scatter in the raw data, as can be seen by the wide range in diversity values at discrete levels of TOC (Fig. 5). Among the possible reasons for such variability in the data are the effects of key environmental factors. For instance, salinity below 18-20 PSU is considered to be a threshold affecting the structure of the benthic assemblages in lagoon habitats (Giangrande et al., 1984; Gravina et al., 1988; Lardicci et al., 1993, 1997, 2001). In our dataset, salinity below this threshold was mainly found in Cabras (Fig. 2b), which also was the lagoon with fewer stations (17% of the total). However, an analysis of stations at salinity <20 PSU showed varying TOC contents and $E(S_{10})$ and H' values, and no correlation between salinity and diversity measures. Other major factors, which can influence both salinity gradients and diversity levels in Mediterranean coastal lagoons, include the water residence times (WRTs) and the confinement of the stations (Remane, 1934; Guélorget and Perthuisot, 1992). As an example, in the Cabras lagoon impoverished benthic communities occur in areas characterized by accumulation of cohesive sediments ($< 8 \mu\text{m}$ grain size particles), capable of binding organic carbon, which is related to the highest WRTs (Magni et al., 2008a, [in press](#)). Moreover, differences in the size of the lagoon (Abele and Walters, 1979) and its degree of connection with the sea (the “island

theory”; McArthur and Wilson, 1967), as well as the number of habitats within each lagoon
355 (Tagliapietra and Volpi Ghirardini, 2006), can influence benthic diversity and thus also serve as a
source variability in our merged dataset. Finally, we should not rule out methodological and
analytical differences between the various studies that produced data for the present analysis, even
though there was an effort to account for such differences where possible (see Sections 2.2 and
2.3).

360 The TOC thresholds found in this study matched (10 mg g^{-1}) or approximated relatively well
($28 \text{ vs. } 35 \text{ mg g}^{-1}$) those reported by Hyland et al. (2005). The difference in the upper TOC
threshold, i.e. a more restricted intermediate range in this study (i.e. TOC from about $10\text{-}28 \text{ mg g}^{-1}$),
may have various explanations. For instance, in contrast to macrotidal estuaries which
represented about one third of the stations in Hyland et al. (2005), the present lagoons are
365 enclosed systems characterized by reduced hydrodynamics and low water exchange (Tagliapietra
and Volpi Ghirardini, 2006; Como et al., 2007; Magni et al., in press). The latter conditions may
increase the risk of hypoxic/anoxic conditions, sulphide development and massive kills of benthos
and fish (Gray et al., 2002; Lardicci et al., 1997; Magni et al., 2008b). For this reason, it seems
reasonable to think that a relatively lower value for the upper TOC threshold is because the
370 stations with high TOC in these lagoons also have co-occurring high levels of other stressors (due
to the long history of human activities in these systems) which are knocking out a larger
percentages of even some of the heartier species. As an example in the Cabras lagoon, Magni et
al. (2005b) showed that peaks in acid volatile sulphide concentrations in summer corresponded to
a major impoverishment of macrobenthos irrespective of seasonal change in sedimentary organic
375 matter concentration, highest between the end of summer and autumn. We also acknowledge that
differences in the composition and bioavailability of organic matter can play an important role in
the derivation of TOC thresholds for assessing risks of benthic impacts. For instance, Pusceddu et
al. (in press) suggested that the biopolymeric carbon fraction (BPC, i.e. protein, carbohydrate and
lipid pools) of sediment organic matter, positively correlated with TOC, is a more sensitive proxy

380 of benthic trophic status than the total carbon pool, due to its higher systematic variability and
different bioavailability along a gradient of organic enrichment. In particular, the authors
identified a critical threshold in BPC concentrations in the sediment of $> 2.5 \text{ mg C g}^{-1}$, being
associated with a bioavailable fraction $< 10\%$, at which benthic consumers may experience mostly
refractory organic carbon. This knowledge was unattainable in the present study because our
385 dataset did not include such measurements. However, if we assume a bioavailable fraction less
than about 10% in eutrophic sediments, as proposed by Pusceddu et al. (in press), our upper TOC
threshold of 28 mg C g^{-1} , being mostly refractory but still indicative of high risks of benthic
impact, would be then rather consistent with a BPC value of about 2.5 mg C g^{-1} . On a related note,
it seems to be of minor importance which of the two lower (10 or 11 mg g^{-1}) and upper (28 or 29
390 mg g^{-1}) thresholds identified in our study were used to define the three TOC ranges (see Section
3.2 and Table 2). Thus, the present results demonstrate the robustness and global applicability of
the TOC thresholds identified originally by Hyland et al. (2005) and confirms TOC as a good
proxy indicator for the rapid assessment of environmental conditions potentially harmful to the
benthos. However, it is important to note that these thresholds should not be considered as
395 absolute values, nor that we are assuming a causal relationship between TOC content itself and
diversity reduction. In fact, the premise of such an approach is that because TOC tends to
correlate with factors causing ecological stress (e.g. low dissolved oxygen, high ammonia and
dissolved sulphide, chemical contamination of sediments; Gray et al., 2002; and review by
Hargrave et al., 2008), then TOC, in turn, may serve as a simple screening-level indicator, or
400 symptom, of such stress (Hyland et al., 2005).

4.3 Lagoon comparison and implications for benthic monitoring and ecological quality assessment of Mediterranean coastal lagoons

A comparison among lagoons showed that there was a general pattern of decreasing
405 diversity from low to high TOC ranges (Table 4). $E(S_{10})$ and H' for all lagoons combined

averaged 5.0 and 2.8 in the low TOC range, 4.3 and 2.3 in the intermediate range, and 3.3 and 1.7 in the high range, respectively. This general pattern was consistent across most individual datasets, though there were some differences among lagoons. For example, there were a few Orbetello stations with low diversity and very low TOC values ($<4 \text{ mg g}^{-1}$) and some Venice stations with a high diversity and very high TOC values ($>40 \text{ mg g}^{-1}$) (Fig. 5). Such cases may be due to inherent data uncertainty as discussed above (Section 4.2), while the latter stations were consistently found to contain a large fraction of refractory organic carbon (Frangipane, 2005). Besides this inconsistency, however, the Venice lagoon showed an overall decreasing diversity from low to high TOC ranges (Table 4). Also the Cabras lagoon, which had the least number of stations and was not represented by stations in the low TOC range, showed a decreasing mean diversity from the intermediate to the high TOC range (Table 4). The main exception to this general pattern was Orbetello, which showed similar mean diversity values at low and intermediate TOC ranges. It should be mentioned here that the Orbetello lagoon experienced an alternate series of dystrophic impacts (Lardicci et al., 1997) and recoveries of the benthic communities (Lardicci et al., 2001) over the past fifteen years. While in this study we did not account for temporal changes, a separate analysis of the 1994 (dystrophy) and 1999 (recovery) Orbetello datasets showed a tendency toward higher diversity values of the latter dataset across the whole TOC range (not shown). Thus, the merging of the two datasets may have hidden any separately consistent pattern within the Orbetello lagoon study. However, also in the case of Orbetello, an analysis of the merged dataset showed again the lowest diversity in the high TOC range.

Overall, this study provides a good example from a relatively large dataset of the levels and ranges of benthic diversity which can be encountered in Mediterranean coastal lagoons. The popular H' diversity index has been used for direct comparisons (e.g. Zettler et al., 2007; Bigot et al., 2008; Blanchet et al., 2008) and recently integrated with other biotic indices (Muxika et al., 2007) as tools for assessing ecosystem condition within the framework of the European Water

Framework Directive (WFD; 2000/60/EC). As an example in a Baltic Sea study, H' was given an absolute scale composed of five classes associated with the different ecological quality (EcoQ) status categories proposed for the WFD, and ranging from $H' > 4$ to $H' < 1$ for “high” and “bad” EcoQ status, respectively (Zettler et al., 2007). The authors showed significant positive correlations between H' and salinity, resulting in a decreased EcoQ with decreasing salinities, thus making this index inappropriate to assess the EcoQ in systems with strong salinity gradients. According to the above scale, 7.4%, 33.2%, and 37.8% of our stations would be classified as “bad”, “moderate,” and “poor”, respectively (in total: 274 out of 349 stations), while only 19.2% and 2.3% of the stations would be classified as “good” and “high” respectively (in total: 75 out of 349 stations). Since the WFD requires that both coastal and transitional waters should achieve the ‘Good EcoQ’ status by 2015, up to 78.5% of our stations, simply based on H' , would be classified below this threshold, thus in the need of remediation measures. However, these results could be related to the inherently reduced (low) number of species occurring in these highly variable and eutrophic systems, which also are exposed to a variety of stressors from multiple human uses. Thus it is suggested that the use of diversity measures alone may be not appropriate to assess the ecological quality of Mediterranean coastal lagoons (see also Munari and Mistri, 2008), unless different thresholds are defined (Chainho et al., 2008). We demonstrate the utility of diversity measures in the context of a conceptual stressor-response framework, such as the P-R model for organic enrichment, with corresponding thresholds for assessing the biological significance of increasing organic enrichment of sediments and associated stressors. While not a measure of causality, the TOC thresholds developed here should serve as a general screening-level tool for evaluating the likelihood of reduced benthic diversity within different ranges of TOC in these eutrophic lagoonal systems.

This study also reinforces the importance of reliable and accurate taxonomy as a starting foundation for the integration of diversity measures and other benthic indicators into comprehensive data sets for large-scale management purposes (Magni et al., 2005a). It also

highlights the value of collaborative regional programmes to help develop consistent and comprehensive sets of indicators and processes that provide a basis for understanding the unique properties of specific ecosystems, such as Mediterranean coastal lagoons (Magni, 2003; Draredja et al., 2006; Cognetti and Maltagliati, 2008; Magni et al., 2008a). This initiative, which began in October 2004 (Magni et al., 2005a), is an important step in this direction and provides a valuable assessment and validation of previously published TOC thresholds (Hyland et al., 2005) as a general screening-level indicator for evaluating the likelihood of reduced sediment quality and associated bioeffects in these systems.

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