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## Trophic state, ecosystem efficiency and biodiversity of transitional aquatic ecosystems: analysis of environmental quality based on different benthic indicators

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Estuaries and coastal lagoons are characterized by a strong spatial and temporal variability of physicochemical characteristics and productivity patterns. In these environments, the magnitude and direction of the ecological responses to inorganic nutrient increase (i.e. eutrophication) are difficult to predict. In the framework of the project, New Indicators of Trophic state and environmental quality of marine coastal ecosystems and transitional environments (NITIDA), we analysed benthic indicators of trophic state, ecosystem efficiency, and environmental quality in four different transitional environments. The trophic state of the sediments was assessed in terms of quantity and bioavailability of sediment organic C pools; ecosystem efficiency was determined in terms of the prokaryote efficiency in exploiting enzymatycally degraded organic C; environmental quality was determined in terms of meiofaunal diversity. Here, we provide a synopsis of the results obtained and a meta-analysis of the scores assessments obtained using the different ecological indicators of environmental quality and demonstrate that trophic state, ecosystem efficiency, and biodiversity in transitional ecosystems are closely linked. We conclude that the assessment of the environmental quality of transitional ecosystems should be based upon a battery of trophic state indicators and 'sensors' of ecosystem functioning, efficiency, and quality.

*Keywords*: Trophic state; Ecosystem efficiency; Biodiversity; Transitional aquatic ecosystems; Environmental quality; Benthic indicators

#### 1. Introduction

Marine sediments can be considered as 'recorders' of the biological processes occurring in the overlying water column from which they receive valuable amounts of material [1]. Therefore, the analysis of the benthic compartment can provide useful information about the whole trophic state of a given aquatic ecosystem [2].

In recent years, increasing attention has been paid to the assessment of the trophic state of aquatic sediments by investigating its biochemical composition in terms of protein,

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carbohydrate, and lipid contents [3]. These three biochemical classes of organic compounds (generally referred to as biopolymeric C, BPC) [3] all together account for 30–70% of total organic carbon and represent a quite reactive fraction of sedimentary organic carbon [2]. In fact, similar biopolymeric organic carbon contents may be characterized by different contributions of each single component, which, in turn, depend upon the system's efficiency in removing (mobilizing) labile rather than refractory molecules.

Shallow, productive, and sheltered marine coastal systems, such as estuaries and coastal lagoons, are generally characterized by a strong spatial and temporal variability of physicochemical characteristics and productivity patterns [4]. In these environments, the magnitude and direction of the ecological responses to inorganic nutrient increase (i.e. eutrophication) are difficult to predict. Pelagic and benthic compartments of shallow marine ecosystems are tightly coupled, so that responses to nutrient enrichment could be seen also as changes in the trophic state of the benthic environment. Extending Nixon's concept of eutrophication from increasing organic supply to the benthos to the accumulation of organic C in the sediment [5], we could assume the latter as an effective indicator of the trophic state of marine coastal environments.

Due to the conservative nature of total organic C in the sediment [6], changes in benthic trophic state could be more evident in terms of organic matter biochemical composition and lability rather than in terms of its concentrations [7]. A universally accepted method to distinguish between the labile and refractory fractions of sedimentary organic matter is not yet available. Biopolymeric organic carbon has often been used as an estimate of the labile fraction of organic C, but it has been demonstrated that only a minor fraction of biopolymeric C in marine sediments is actually bioavailable (i.e. enzymatycally digestible) [8], thus representing only a weak descriptor of the trophic state of sediments. Such a weakness could be more evident in estuaries and coastal lagoons that accumulate huge amounts of organic C, functioning as a sink of organic detritus [9].

The high prokaryote density in marine sediments [10–12] suggests that heterotrophic prokaryotes play a major role in sediment organic matter degradation processes [13] and transfer to higher trophic levels [14–16]. The relative importance of prokaryote-mediated organic matter degradation processes and consequent bacterial assimilation depend upon the quality and quantity of the available substrates and, therefore, upon the trophic state of the system. Hydrolysis rates of organic substrates appear faster than assimilation rates when the organic loads increase over certain threshold levels (eutrophicated conditions). This determines an evident malfunctioning of the microbial loop, finally leading to a further accumulation of unexploited organic material [17–20]. Such alterations induce modifications of the trophic state and ecological integrity. Following such scientific evidence, the use of indicators based on prokaryote abundance and activity for the assessment of the ecological integrity of a given system appears appropriate to detect rapid environmental changes.

Due to the short generation time, the high sensitivity to any changes in environmental conditions, and lack of pelagic larval dispersion, meiofauna (body size ranging between 40 and 500  $\mu$ m) represent an increasingly common tool for environmental monitoring [21, 22]. For their characteristics, meiobenthic organisms display a rapid response (in terms of abundance and diversity) to environmental alterations, stress, or disturbance conditions [23–29] and, in this sense, can integrate information based on the analysis of the macrobenthic compartment.

The assessment of the environmental quality of aquatic ecosystems, since the Water Framework Directive (WFD), is one of the major objectives of applied aquatic ecology in Europe. Considerable experience gained in the environmental quality assessment of freshwaters has generated a number of indicators based on water column variables and on biological indicators. Such experience has been targeted only marginally to transitional environments, in which, traditionally, most studies and monitoring programs have been focused on the quality of waters.



Figure 1. Study area and stations location.

In order to provide a scientific contribution to the possible implementation of the Water Framework Directive, we analysed benthic indicators of trophic state and environmental quality in four different transitional environments.

Here, we provide a comparative analysis of the scores obtained using the different indicators. The aim of this comparison is to highlight the need of assessing the environmental quality of transitional ecosystems on the basis of both (trophic) state indicators and 'sensors' of ecosystem efficiency and quality.

#### 2. Methods

#### 2.1 Study areas

The Venice lagoon has a mean depth of c. 1 m and communicates with the northern Adriatic Sea through three main inlets: Chioggia, Malamocco, and Lido. The lagoon exchanges c. 60% of its waters at any tidal cycle (c. 12 h) and receives sewage from the cities of Venice, Mestre, and the Marghera industrial districts (figure 1A).

The Goro lagoon, the southernmost basin of the wider Po deltaic system (average water depth of 1.5 m), is subjected to continuous natural subsidence phenomena and large anthropogenic loads. The basin is connected to the sea by means of both natural and artificial canals and, during summer, may experience sub-oxic/anoxic conditions with dystrophic crises related with macroalgae blooms (*Ulva* sp. and *Gracilaria* sp.) (figure 1B).

The Lesina lagoon, characterized by shallow depths (on average 0.8 m depth), is connected to the sea through two channels and receives freshwater from two minor rivers. Lesina lagoon is experiencing an increasing eutrophication and occasional dystrophic crises related to *Valonia utricularis* blooms. Large parts of the lagoon are also covered by *Zostera noltii* and *Ruppia* sp. (figure 1C).

The Marsala lagoon is connected to the sea by means of two large channels allowing a large mixing with sea waters [8]. No major continental inputs are present. The average water depth is c. 1 m, and most of the lagoon is characterized by a seagrass meadow (*Posidonia oceanica*) (figure 1D).

#### 2.2 Sampling and investigated variables

Sediment samples were collected through manual coring in one representative station of the lagoons of Venice (June 2004), Goro (May and July 2004), and Lesina (May and July 2004) (figure 1). Also, the Marsala lagoon was investigated for some variables. In this study, only the mean annual values (±standard error) are reported.

At each lagoon, surface sediments (0–1 cm) were analysed for total and digestible protein and carbohydrate and total lipid and phytopigments [30], prokaryote abundance, biomass and C production [31], extracellular enzymatic activities [14], meiofaunal abundance, and diversity (i.e number of taxa) [32]. The autotrophic fraction of sediment organic C (as the fraction of phytopigments to biopolymeric C) and the concentrations of bioavailable organic C (as the sum of digestible proteins and carbohydrates) were used as combined indicators of the benthic trophic state [30].

Ecosystem efficiency was defined as the prokaryote C conversion efficiency, which enables one to estimate the efficiency of energy transfer towards higher levels. The prokaryote C conversion efficiency was calculated as the fraction of enzymatycally degraded organic C incorporated into prokaryote biomass. Enzymatically degraded organic C was determined by converting the extracellular enzymatic activities ( $\mu$ mol g<sup>-1</sup> h<sup>-1</sup>) into C equivalents using 72  $\mu$ g C  $\mu$ mol<sup>-1</sup> as the conversion factor. Prokaryote turnover of biopolymeric C was calculated as the ratio between prokaryote C production and the biopolymeric C sediment concentrations. Environmental quality was assessed using the number of meiofaunal taxa as a determinant [32].

#### 3. Results and discussion

#### 3.1 Benthic trophic state assessment

In monitoring programs, the trophic state of aquatic ecosystems is typically assessed by means of descriptors determined in the detrimental effects of eutrophication, including dystrophic crises, generally begin and develop in the benthic compartment.

In this study, we combined two different tools for assessing the trophic state of benthic environments of transitional ecosystems: (1) the use of biopolymeric C as a quantitative descriptor of the amount of trophic resources for the benthos [2] and (2) the use of the digestible fraction of biopolymeric C as a proxy of its bioavailability [8, 10].

All the investigated sediments, apart those in the Marsala lagoon, were characterized by concentrations of biopolymeric C exceeding  $5 \text{ mg C g}^{-1}$  (figure 2a) and, according to the threshold levels proposed by Pusceddu and Danovaro [33], should all be ranked as eutrophic. This would mean that, using the biopolymeric C concentrations as the unique indicator, all these systems should be ranked as eutrophic. However, this is not the case. The four investigated systems are, in fact, transitional environments and as such, being fuelled either by freshwater and marine coastal production, are 'naturally' eutrophic. Therefore, this approach appears unable to describe accurately the differences in the trophic state of the systems.

In a previous study, it has been reported that the biopolymeric C content of coastal sediments is inversely related to the fraction of sediment organic matter associated with fresh algal material [8]. Therefore, the combination of these two proxies was proposed as a synthetic descriptor of benthic trophic state was [33] (table 1). The application of the thresholds levels of biopolymeric C proposed previously to the sediments of the four transitional systems investigated in the present study (table 2) led all the four systems, apart from the Marsala lagoon, to score as eutrophic, so no differences appear to exist between these systems.



Figure 2. Biopolymeric and bioavailable organic C pools in the sediments of the four investigated lagoons.

Table 1. Threshold levels of the trophic state classification proposed by Pusceddu and Danovaro [33] and the relative scores of the four systems investigated in this study.

Trophic state	Biopolymeric C (mg $g^{-1}$ )	Autotrophic fraction of biopolymeric C (%)
Oligotrophic (OL)	<1	>15
Mesotrophic (MES)	1–5	8-15
Eutrophic (EU)	>5	<8

Table 2. Relative scores of the four systems investigated in this study.

Area	Biopolymeric C $(mg C g^{-1})$	Autotrophic fraction of biopolymeric C (%)	Score (Pusceddu and Danovaro [33])
Venice	$7.50\pm0.87$	$2.11\pm0.18$	EU/EU
Goro	$12.44 \pm 1.64$	$8.96\pm0.92$	EU/MES
Lesina	$16.21 \pm 1.40$	$5.11 \pm 0.56$	EU/EU
Marsala	$0.20\pm0.04$	$52.76 \pm 8.00$	OL/OL



Figure 3. Relationship between bioavailable organic C in the sediment and the fraction of biopolymeric C associated with phytopigment.

The concentrations of the digestible organic matter in the sediment differed significantly among the four investigated systems (figure 2B). Differences between the four lagoons were as relevant as those observed in terms of the whole biopolymeric C sediment contents and were even more evident in terms of the fraction of the digestible organic material (figure 2C).

In this study, we verified that the amount of digestible organic C in the sediment and the autotrophic fraction of biopolymeric C were negatively correlated (figure 3) indicating that sediments characterized by increasing bioavailable C contents are characterized by an accumulation of organic matter of detrital or heterotrophic nature. These results suggest that the amount of digestible biopolymeric C is a sensitive descriptor of the differences in trophic conditions of transitional environments.

This funding confirms that the benthic eutrophication process cannot be simply viewed as the result of enhanced inputs from organic C as a whole but, further, as an exacerbated alteration of benthic C cycling linked to the accumulation of primary organic matter, labile (digestible) compounds, and associated benthic metabolism [8, 34].

#### **3.2** Evaluation of ecosystem efficiency

Coastal lagoons are characterized by high primary productivity able to sustain locally high secondary production [4]. In addition, large detrital organic pools can accumulate in the sediments, and because of its mainly refractory composition, only a minor fraction of this detritus is directly available to the consumers [8].

In transitional environments, most studies have focused on the distribution of metazoans along the environmental gradients (i.e temperature and salinity), whereas prokaryotes, the main actors of the internal nutrient cycling [35], have been largely neglected.

However, in these systems, characterized by high organic C levels, the hydrolysis of the organic substrates is a key process which help to understand a possible malfunctioning of the microbial loop [35]. Therefore, the assessment of the degradation activities mediated by prokaryotes and the incorporation of mobilized organic C into prokaryote biomass are key variables useful for addressing the levels of ecosystem efficiency.

We report here that the prokaryote turnover of biopolymeric C and the fraction of enzymatycally degraded organic C incorporated into prokaryote biomass differed significantly at three of the four investigated lagoons (figure 4A and B). The pattern in prokaryote efficiency



Figure 4. Relationships between prokaryote C incorporation efficiency, biopolymeric C turnover, and its autotrophic fraction.

in exploiting organic C follows the pattern in the accumulation of digestible organic matter in the sediment. Moreover, moving from less to more eutrophic systems, the efficiency in the C incorporation rates of benthic prokaryotes increases, whereas the fraction of primary organic matter that accumulates in the sediments decreases (figure 4C). This can be interpreted as the tendency of highly eutrophic systems (such as the Venice lagoon) to shift from a general autotrophy to a net heterotrophy. In addition, the increase in prokaryote efficiency in exploiting organic C in eutrophic systems is consistent with the tendency of organic-C enriched systems to shift towards dystrophic conditions.

These results indicate that the occurrence of dystrophic crises cannot be considered only a consequence of increased primary production rates but must be considered also as the result of a shift of the C cycling into the system from the grazing food chain into the microbial loop.

#### 3.3 Descriptors of environmental quality

Meiofaunal assemblage is a collective indicator of environmental quality, able to display different responses to different kinds of anthropogenic disturbance [36]. This makes the use of

meiofauna preferred over other benthic components, especially when the disturbance source has not been identified [37].

Recent investigations have pointed out that the richness of meiofaunal taxa has the potential to provide straightforward identification of the effects of differential alterations of the ecosystem [38]. Diversity and richness of taxa are generally lower in polluted and stressed environments, due to the disappearance of certain more sensitive taxa (e.g. ostracods, gastrotriches, hydrozoans, tardigrades, etc.) and the overwhelming abundance of tolerant taxa (e.g. nematodes).

Eutrophicated systems, being characterized by a huge accumulation of organic C in the sediment and, as such, being exposed to dystrophic crises, can be expected to be inhabited by a limited number of meiofaunal taxa, whose number should tend to decrease as the organic C content in the sediments increase over the levels of dystrophic conditions.

In this study, the number of meiofaunal taxa ranged between 4 and 10, and displayed clear differences between the three lagoons (figure 5A), with the Venice lagoon being characterized by the lowest number of taxa.

In a recent study, Danovaro *et al.* [32] proposed a classification of the environmental quality of sediments based on the number of meiofaunal taxa (table 3). According to this classification, the three investigated lagoons all scored between moderate impact and sufficient conditions, with the latter being observed only in the Lesina lagoon. However, when looking at the seasonal variability of these scores (measured in the Goro and Lesina lagoons only; figure 5B) we observed that the environmental quality typically decreased from spring to summer, concurrently with increased levels of organic C accumulation and oxygen consumption. This result indicates that the number of taxa is a useful and reliable tool for the assessment of the benthic environmental quality of transitional environments.



Figure 5. Meiofaunal taxon richness in three of the four investigated lagoons (A) and seasonal variations of the number of taxa in the Lesina and Goro lagoons (B).

Number of taxa	Environmental quality	Taxa present
≤4 taxa	Strong impact	Nematodes, Copepods, Polychaetes, Oligochaetes
From 4 to 7 taxa	Moderate impact	All the above, plus Gastrotrichs, Amphipods, Bivalves, Ostracods, Turbellarians
From 8 to 11 taxa	Sufficient environmental quality	All the above, plus Kinorhynchs and Cnidarians
From 12 to 16 taxa	Good environmental quality	All the above, plus Isopods, Tardigrades, Acaria, Gastropods, Hydrozoans
≥16 taxa	Excellent environmental quality	All the above, Cumaceans, Nemerteans and others

 Table 3.
 Classification of environmental quality of sediments based on biodiversity (as the number of meiofaunal taxa; [32]).

Table 4. Synopsis of scores gathered using the three descriptors adopted in this study.

Lagoon	Trophic status	Ecosystem efficiency	Environmental quality
Venice	Eutrophic	Rapid organic C turnover, heterotrophic	Moderate impact
Goro Lesina	Meso-Eutrophic Eutrophic	Slow organic C turnover, autotrophic Intermediate organic C turnover, auto/heterotrophic	Moderate impact Sufficient
Marsala	Oligotrophic	NA	NA

NA: not available.

#### 3.4 Synopsis and conclusions

Our results suggest that the whole quality of transitional environments cannot be achieved using a unique indicator. Biological communities of transitional environments, being subjected to a wide spatial and temporal variability of physical–chemical conditions, generally respond quickly to any environmental change. Therefore, the sensitivity of descriptors should fall within the range of these adaptive responses. For this reason, neither the whole amount of sediment organic C (that has been demonstrated to be rather conservative) [8] nor the macrofaunal component could provide a fast-response indication of ecosystem alteration.

The battery of indicators we tested in this study provided reliable results in distinguishing between the investigated systems in terms of trophic conditions, ecosystem efficiency and environmental quality. The synopsis of the quality of the ecosystems attributes carried out using the scores provided by the three descriptors indicates that they do not always match; taken together, they provide an overall judgement of environmental quality (table 4).

The goal of assessing the environmental quality of transitional systems is basically difficult because of the internal complexity of these ecosystems. More work and data analysis are needed to refine the use of the new indicators of trophic state and environmental quality that we tested in this study. The results we provided here proved that they can be useful, at least if considered in a multi-level battery of ecological indicators.

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