




Article

# Conservation Paleobiology as a Tool to Define Reference Conditions in Naturally Stressed Transitional Settings: Micropaleontological Insights from the Holocene of the Po Coastal Plain (Italy)

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**Abstract:** The key role of paralic environments as providers of ecosystem services, associated with their increasingly threatened state, led to the definition of international water management policies aimed to improve ecological quality status (EcoQs). Restoration actions rely on the definition of reference conditions, which is a particularly challenging task in naturally stressed transitional environments. In the present work, we apply the diversity index  $\text{Exp}(H'_{bc})$  on benthic foraminifer assemblages from two anthropogenically unimpacted transitional to coastal Holocene sediment successions of the Po coastal plain, in order to assess past EcoQs (PaleoEcoQs). Ostracod ecological groups provided detailed insights on naturally stressful paleoenvironmental conditions. We show that “poor” to “moderate” PaleoEcoQs are recorded by biological indicators at reference conditions under fluctuations of chemical-physical parameters and organic matter enrichment. We emphasize the importance of a site-specific paleobiological approach, as significant differences in diversity occur even on a short spatial scale. This study illustrates that early to mid-Holocene sediment successions resulted to be appropriate for conservation paleobiological purposes, providing a high-resolution paleoecological record under the influence of the Holocene sea-level rise in analogy with the present-day global change.

**Keywords:** conservation paleobiology; benthic foraminifers; ostracods; Holocene; Po coastal plain; PaleoEcoQs

## 1. Introduction

At the border between the continental and the marine realm, transitional settings such as lagoons and estuaries are known for their fragile equilibrium [1]. Enrichment in nutrients, heavy metals, and organic contaminants are often determined by the increasing anthropogenic pressure of the last centuries in the form of aquaculture, agricultural, industrial, and commercial activities. The key role of ecosystem services provided by transitional water habitats led to the definition of national and international conservation guidelines back to ca. 50 years ago (e.g., Federal Water Pollution Control Act, USA, 1972; [2]). More recently, European countries have implemented the European Water Framework Directive (WFD – 2000/60/EC) that aims to achieve “good” or “high” ecological quality status (EcoQs) within 2020 [3]. The definition of reference conditions is essential to perform a correct evaluation of the EcoQs, and implement effective actions for the restoration of potentially threatened coastal and transitional water bodies. Specifically, reference conditions are biological quality elements for a

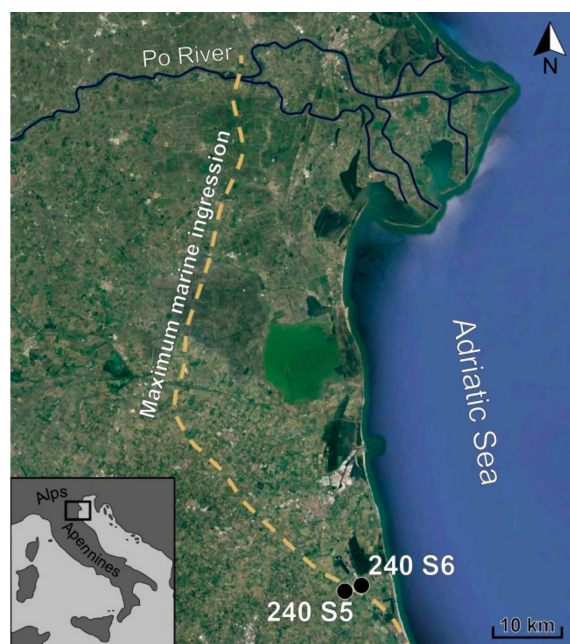
type-specific water body at high ecological status, whereas the ecological status includes “biological, physico-chemical and hydromorphological elements” [4]. Reference conditions should reflect the natural variability of the system, but transitional settings are naturally stressed environments subject to strong fluctuations of many chemical-physical parameters, such as water salinity, turbidity, nutrient, and oxygen concentrations. Biotic communities are dominated by taxa adapted to tolerate such intense, rapid excursions of environmental parameters. Nevertheless, communities with similar features in terms of abundance, structure, and composition also occur in anthropogenically impacted areas. The resulting difficulty of discerning natural stressors from anthropogenic impacts in transitional settings determines the so-called “Estuarine Quality Paradox” [5,6].

An accurate definition of reference conditions and appropriate calibration of boundary thresholds between EcoQs could help in the estimation of anthropogenically induced conditions, preventing transitional environments from appearing more impacted than they really are [7,8]. Ideally, reference conditions should be determined at an almost undisturbed site or, in the second instance, on historical data [4]. Conservation paleobiology can provide an appropriate approach to the definition of reference conditions, as it allows to explore the biotic response to both anthropogenic and natural stressors applying “theories and analytical tools of paleontology to biodiversity conservation” [9,10]. Even though most marine conservation biologists considered a centennial or millennial time-scale appropriate for assessing climate change issues only, such paleontological perspective demonstrated to be essential in the definition of (paleo-)environmental ranges of variation in response to a set of environmental stressors [11], also of natural origin [12].

Foraminifers are part of the benthic invertebrate fauna, indicated as a biological quality element for the definition of EcoQs in coastal and transitional waters [4]. Abundant literature demonstrated the efficiency of this group for (bio-)monitoring purposes (e.g., [13,14]). Guidelines for environmental monitoring through benthic foraminifers were produced for the marine realm [15–17], and the developed biotic indices have been currently testing [18–20]. Nevertheless, only a few works dealt with the same purpose in transitional waters, and essentially focused on modern environments in Italy [21], located on both the Adriatic and the Tyrrhenian coasts. The reference dataset of Italian transitional waters [21] substantially covers environmental conditions for microtidal systems, from marsh to outer lagoon, and related chemical-physical parameters, such as salinity, pH, freshwater contributions, dissolved oxygen, organic carbon, and trace metals. Environmental changes in these areas occur at a local scale, driven by natural (e.g., seasonality and degree of confinement; [22]) and anthropogenic forcings (e.g., eutrophication and heavy metal contaminations; [23,24]). The high environmental coverage of the modern dataset ensures a reliable comparison with the early to mid-Holocene transitional successions of the Po coastal plain, where similar conditions are commonly recorded [25–27]. Ostracods also fall within the huge group of the benthic invertebrate fauna, and share with benthic foraminifers many desirable properties, that make these taxa ideal for integrated paleoecological reconstructions, particularly in transitional settings where strongly reduced salinities may prevent the development of foraminifer communities [28,29]. Even though the response of brackish water ostracods to anthropogenic pollution has not been extensively investigated (e.g., [30,31]), they are excellent proxies of freshwater and organic matter inputs, water turbulence, and permanence in a continuum from continental to back-barrier settings [32–34].

In this respect, the Holocene back-barrier succession of the Po coastal plain (N Italy, Figure 1) can represent a valuable resource to investigate pre-impacted conditions in transitional environments. The abundance of threatened coastal water bodies in this area [35–38] makes such a purpose even more urgent to plan efficient restoration actions. Relationships between past and present-day environmental conditions can be efficiently evaluated through a conservation paleobiology perspective, most commonly achieved through invertebrate macrofauna (e.g., mollusks and corals; [12,39]). In the North Adriatic area, such an approach successfully outlined the long-term resilience and persistence of late Pleistocene-Holocene marine molluscan assemblages to major environmental changes under natural forcings in contrast to the abrupt, unprecedented shift in community composition and structure

that occurred in the Anthropocene [40,41]. Seasonal hypoxia, sediment pollution, bottom trawling, and warming events were determined to have a great impact on benthic communities in the last centuries [42–45], but also impacted the sedimentary record affecting the temporal resolution of fossil assemblages [40,46]. Disentangling sedimentological and biological effects on benthic communities appears as a key to quantifying paleoecological changes in a high-resolution chronostratigraphic framework [47]. In this context, ecosystem fluctuations can be effectively assessed from continuous archives such as back-barrier sediment successions [48,49], where the benthic meiofauna is a proxy of PaleoEcoQs of growing importance, thanks to its abundance, biological properties, and preservation potential [50].



**Figure 1.** Location of cores 240 S5 and 240 S6. Maximum marine ingression from [51]. Satellite image: © 2020 Google.

In the present work, we evaluate if reference conditions can be assessed within pre-impacted transitional successions applying ecological indices routinely used to detect anthropogenic pollution in modern environments on benthic meiofauna. We reconstruct the paleoecological quality status (PaleoEcoQs) from benthic foraminifers within two Holocene paralic successions (*sensu* [52]) of the Po coastal plain. The ostracod record is integrated to obtain a more detailed picture of paleoenvironmental conditions under natural stressors from a separate line of evidence.

## 2. Geological Setting

The Holocene succession of the Po coastal plain is developed atop of late Pleistocene alluvial sediments and consists of a ca. 30 m-thick transgressive-regressive wedge [53,54]. In response to the Holocene transgression, the development of a wave-dominated estuary is recorded by the superposition of back-barrier to offshore deposits (*i.e.*, retrogradational stacking pattern facies), followed by deltaic sediments typifying the progradation of the Po coastal plain after the sea-level stabilization [55,56].

Thick back-barrier successions are mostly developed in proximity to the maximum shoreline ingression, located up to ca. 26 km west from the modern shoreline in the northern part of the study area, whereas an inland migration of a few km only occurred in the southern portion [51] (Figure 1). Lower transgressive intervals are made up of swamp and lagoon fine-grained sediments, enriched in organic material, testifying the first phases of marine ingression that led to the submersion of wide lowland areas where reducing and sediment-starved conditions locally occurred [56]. Swamp-lagoon alternations often recorded within transgressive successions reflect localized progradational episodes

connected to autogenic processes under a predominant allogenic-controlled sedimentation [25]. Lagoon sediments with sand intercalations and coarse-grained transgressive barrier sands at seaward locations are indicative of less restricted conditions developed at peak transgression (7.7 to 7.0 cal. ky BP; [57]). Inland, the shift to highstand conditions is represented by thick swamp to alluvial plain sediments indicative of lagoon infilling, whereas beach ridge and delta front sands at seaward locations express coastal progradation [27].

No anthropogenic influence is recorded in the Holocene transgressive and early regressive sediment succession of the Po coastal plain, where transitional to coastal paleoenvironments are considered as pristine and were used to define geochemical background values at a regional scale [58–60]. Specifically, Ref. [36] defined natural background values for the southern Po coastal plain including geochemical data from core 240 S5, reported in [25]. Natural enrichment in Cr and Ni determined by the contribution of mafic and ultramafic rocks cropping out extensively in the Po River drainage basin is restricted to the sediments of the northern Po coastal plain, whereas low values are recorded in the southern coastal plain drained by NE Apenninic rivers [61].

### 3. Materials and Methods

Cores 240 S5 and 240 S6 were recovered in the southern Po coastal plain, less than 10 km inland from the modern shoreline and 2.6 km distant from each other (Figure 1). Both cores are 40 m long and were drilled by wire-line perforation, which guaranteed a very high recovery percentage (>90%). Sedimentological characteristics of the cores, including accessory components such as macrofossils and vegetable debris are described in [62]. Further details on core 240 S5, including geochemical and qualitative micropaleontological data are reported in [25]. In the present work, we present the micropaleontological record of core 240 S6 and provide more detailed data from core 240 S5 using quantitative benthic foraminifer and semi-quantitative ostracod fauna.

The lower portion of both cores is made up of continental deposits of late Pleistocene age, passing upward to a ca. 15 m-thick back-barrier succession including a suite of swamp to lagoon facies that evolve into shallow marine and coastal sediments in core 240 S6 only [62]. Subtle changes in the composition of microfossil assemblages within lagoonal intervals are interpreted to reflect short-term paleoenvironmental changes connected to variations in sediment supply at a local scale under a general trend of sea-level rise [25]. Both successions are capped by modern alluvial plain sediments [62].

A total of 129 samples were collected for micropaleontological analysis of core 240 S6, with a thickness of about 1.5 to 2 cm, corresponding to ca. 100 g of sediment. Benthic foraminifer and ostracod fauna was recovered following the standard procedure adopted for sample treatment in core 240 S5 [25] and other reference works dealing with the microfossil content of the Po Plain Holocene successions (e.g., [63]). It involves: (i) oven-drying the samples at 60 °C for at least eight hours; (ii) soaking in water or water + H<sub>2</sub>O<sub>2</sub> (35% vol.) for 24 hours; (iii) wet-sieving at 63 µm; (iv) oven-drying again. Sixty-three samples containing well-preserved and abundant meiofauna were selected for micropaleontological investigations, performed in the sediment fraction >125 µm. The set of 109 samples of core 240 S5 of [25] was reviewed selecting suitable samples for quantitative analyses.

Identification of benthic foraminifers and ostracods followed the original taxonomic descriptions [64,65] and reference works mostly focused on the Mediterranean area as reported in Text S1. When possible, at least 300 well-preserved benthic foraminifer specimens were counted and identified within 41 samples (25 from core 240 S5, 16 from core 240 S6), dividing the residue into small portions with a microsplitter if necessary (abundance data provided in Table S1), otherwise, all the retrieved tests were counted. Relative abundances of ostracods were expressed by four categories (abundant: >30%, common 10% to 30%, scarce 4% to 10%, rare <4%) in a total of 95 samples (54 from core 240 S5, 41 from core 240 S6; Table S2). We considered adult and juvenile ostracods bearing morphological characters that allowed an identification at species level, whereas valves with evident signs of transport or reworking (dark appearance, strong abrasions, fragmented, etc. [66]) were discarded.



To test if natural reference conditions could be assessed by benthic foraminifers, we applied the diversity index proposed by [13] as a proxy for (Paleo-)EcoQs in cores 240 S5 and 240 S6, which represent transitional to marine Holocene paleoenvironments with pristine conditions. The index follows the [67] modification ( $H'_{bc}$ ) of the Shannon-Weaver index of community entropy ( $H'$ ; [68]), which fixes the problem of possibly unobserved species in the case of under-sampling. Then, the exponential of the obtained number ( $\exp(H'_{bc})$ ) allows the shift to a true diversity measure that expresses the number of species with the same abundance required to obtain  $H'_{bc}$  [13]. Considering the paleoenvironmental setting, we applied the classes of EcoQs defined for present-day Italian transitional waters [21]. Guidelines for the determination of EcoQs were established by [13], which recommend the use of living assemblages reflecting real-time environmental conditions, nonetheless dead assemblages that represent a longer time frame show a lower, but still significant, degree of correlation with oxygen content. Even though many studies dealing with (Paleo-)EcoQs are performed on the  $>63 \mu\text{m}$  size fraction, Ref. [13] suggest that the  $>125 \mu\text{m}$  fraction is also adequate due to the negligible information lost, the lower time and taxonomic effort required for the analysis. Fossilizable taxa obtained through sample drying are considered to provide an accurate estimation of (paleo-)environmental conditions to compute (Paleo-)EcoQs, as fragile agglutinated foraminifers preserved by wet-picking do not substantially improve the (paleo-)ecological signal [13].

The Foram-AMBI index was calculated to estimate the stress related to organic carbon concentration on the basis of benthic foraminifer ecological groups with different responses to organic enrichment [15]. Despite the Foram-AMBI being developed for the marine realm, we apply it to the analyzed assemblages to assess the abundance of organic matter sensitive, tolerant, and opportunistic taxa in transitional settings under natural conditions. We used benthic foraminifer groups defined for the Mediterranean area by [17], specifically: GI—sensitive species; GII—indifferent species; GIII—third-order opportunists; GIV—second-order opportunists; GV—first-order opportunists. We computed the  $\exp(H'_{bc})$  and Foram-AMBI indices in benthic foraminifer assemblages including  $>100$  tests, which is considered a suitable threshold value for (paleo-)ecological analyses on assemblages that commonly show low abundances in stressed settings [69,70].

Finally, a detrended correspondence analysis (DCA) was computed on benthic foraminifers showing relative frequencies  $>1\%$  with the aim of exploring the relationship between taxa, assemblages, and the computed ecological indices through paleoenvironmental gradients. DCA is an indirect ordination technique that allows for the assigning of scores to both species and samples in the multivariate space and was successfully applied in back-barrier to shallow marine settings for (paleo-)ecological purposes [71–73]. Here, we apply DCA separately for the benthic foraminifer datasets of core 240 S5 and 240 S6 to visualize the correlation between environmental gradients and ecological indices, plotted as additional variables within a more restricted setting (240 S5) and throughout the transition from back-barrier to shallow marine settings (240 S6). A few taxa characteristic of inner to outer shelf environments (*Cibicidoides lobatulus*, *Asterigerinata planorbis*, *Praeglobobulimina* sp.) possibly transported in the restricted back-barrier setting of core 240 S5 that determined strong distortion of the detected paleoecological gradient were removed. The DCA was computed in the R environment (version 3.6.1, R Foundation for Statistical Computing, Vienna, Austria; [74]) using the vegan package [75].

Ostracods provided detailed insights on paleoecological conditions, particularly in back-barrier sediments, integrating the record of benthic foraminifers. Autoecological data on individual taxa were mostly extracted from [76] and additional references when necessary [77–79], which allowed to obtain insights on water salinity, oxygen, turbulence, and organic matter inputs (Table S3) also within sediment intervals with no benthic foraminifers. Tolerance to pH oscillations and water permanence was also inferred for assemblages with freshwater to low brackish taxa [76], whereas no data are available for brackish, euryhaline, and marine species.

The chronological framework of the two cores is provided by seven radiocarbon dates performed on samples enriched in organic matter and mollusk shells [25,54]—(Table 1). Calibration of conventional

radiocarbon ages was performed with OxCal 4.2 [80], applying the Intcal13 calibration curve or the Marine13, with a  $\Delta R$  value of  $139 \pm 28$  years [81], and a mixed contribution in back-barrier samples.

**Table 1.** List of radiocarbon ages from cores 240 S5 and 240 S6.

Core	Core Depth	Material	Conventional Age	Calibrated $2\sigma$ Age	Calibrated Age Mean (yr BP)	$\sigma$	Dating Laboratory	Reference
240 S5	9.5	Organic clay	5290 $\pm$ 100	6300–5770	6080	110	ENEA (Bologna, Italy)	[62]
240 S5	12.9	Organic clay	6770 $\pm$ 105	7600–7230	7410	90	"	"
240 S5	18.5	Organic clay	10650 $\pm$ 130	12770–12140	12540	150	"	"
240 S5	23.4	Organic clay	12500 $\pm$ 220	15470–13960	14690	390	"	"
240 S5	31.6	Organic clay	19050 $\pm$ 230	23520–22450	22980	280	"	"
240 S6	21.55	Mollusk shell	7384 $\pm$ 45	8037–7856	7958	45	CEDAD (Lecce, Italy)	[54]
240 S6	23.6	Mollusk shell	7358 $\pm$ 45	8013–7843	7932	45	"	"

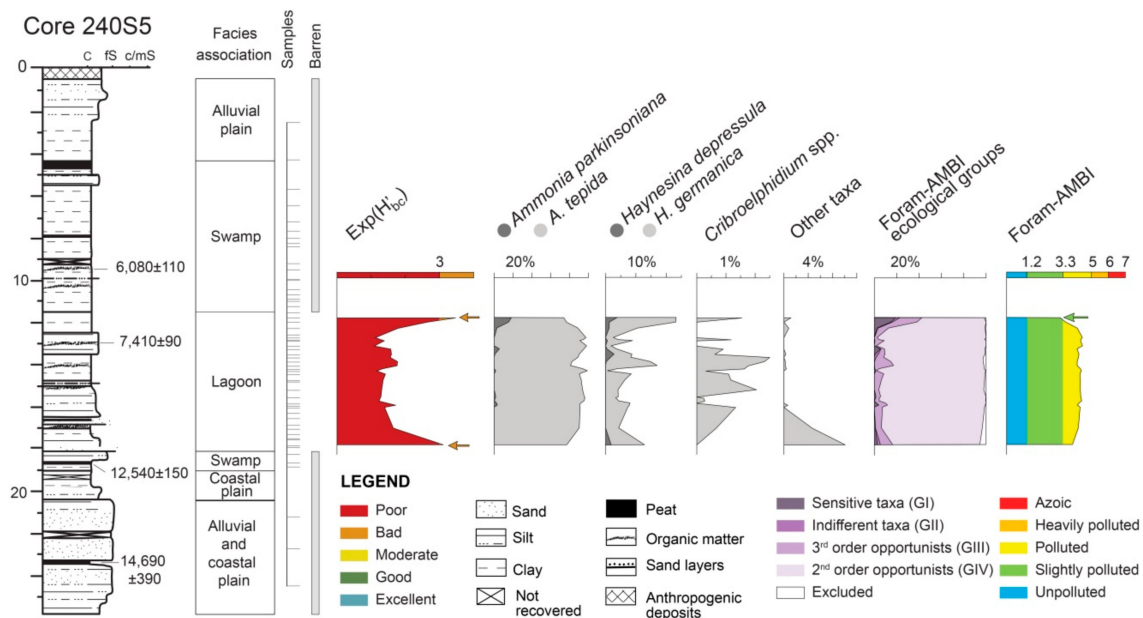
## 4. Results

### 4.1. Benthic Foraminifers

A total of 15138 benthic foraminifer specimens, belonging to 38 taxa, were recorded in the analyzed successions; in particular, core 240 S5 included 7949 tests represented by 19 taxa from the 17.95 to 11.85 m core depth, and 7189 specimens of 24 taxa were recorded from core 240 S6 in the interval between 23.60 and 10.10 m core depth (Table S1).

#### 4.1.1. Core 240 S5

Exp( $H'_{bc}$ ) index showed values  $<3$  in almost all samples, with the exception of the lowermost and uppermost samples (17.95 and 11.85 m core depth) characterized by values slightly  $>3$ . The resulting PaleoEcoQs is classified as “poor” from 17.55 to 11.95 m core depth and as “bad” in the remaining samples (Figure 2, Table S1).

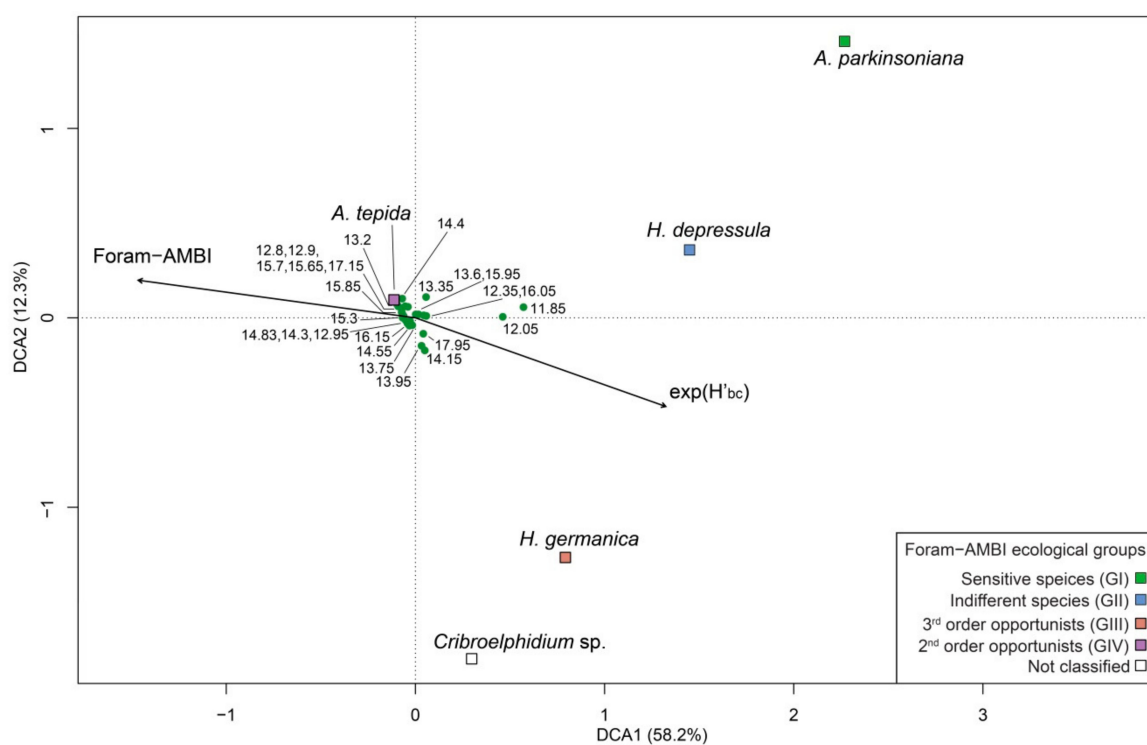


**Figure 2.** Stratigraphy, vertical distribution of benthic foraminifer taxa,  $exp(H'_{bc})$ , and related PaleoEcoQs, Foram-AMBI ecological groups, and Foram-AMBI values of core 240 S5. Ages are reported as calibrated years BP. Samples barren in benthic foraminifers are evidenced. Arrows are colored according to sample classification to improve the graphical visibility of selected samples.

The taxonomic composition of fossil assemblages determined moderately high Foram–AMBI values, mostly between 3.5 and 5, excluding the uppermost sample at 11.85 m core depth that shows a value of 3.2. The most abundant ecological group was represented by second-order opportunist taxa, with an average abundance of 88.3% and reaching peak frequencies >95%. Third-order opportunists (tolerant) taxa were relatively scarce (7.6% on average), whereas indifferent and sensitive species were rare (0.6% and 2.3%, respectively) and mostly recorded in the samples between 12.6 and 11.85 m core depth (Figure 2).

The low diversity of samples from the lagoon interval of core 240 S5 is mostly determined by the strong dominance of *Ammonia tepida* (88.3% on average), associated with lower abundances of *Haynesina germanica* (7.5%). The former, classified as a second-order opportunist, determined the high Foram–AMBI values throughout the succession in association with tolerant species which were mostly represented by *H. germanica*. Other taxa, such as the sensitive *Ammonia parkinsoniana* and the indifferent *Haynesina depressula*, mostly occur with relatively low abundances in the upper portion of the lagoon sediments (Figure 2).

DCA arranged the benthic foraminifer variables in a wedge-shaped distribution (Figure 3), with DCA axis one accounting for 58% of the total variance. Within this relatively short paleoenvironmental gradient, taxa are distributed along axis one in accordance with their degree of tolerance to organic enrichment. Samples with a higher abundance of sensitive species are placed on the right side of the plot. Foram–AMBI and  $\exp(H'_{bc})$  vectors showed a good correlation with axis one.

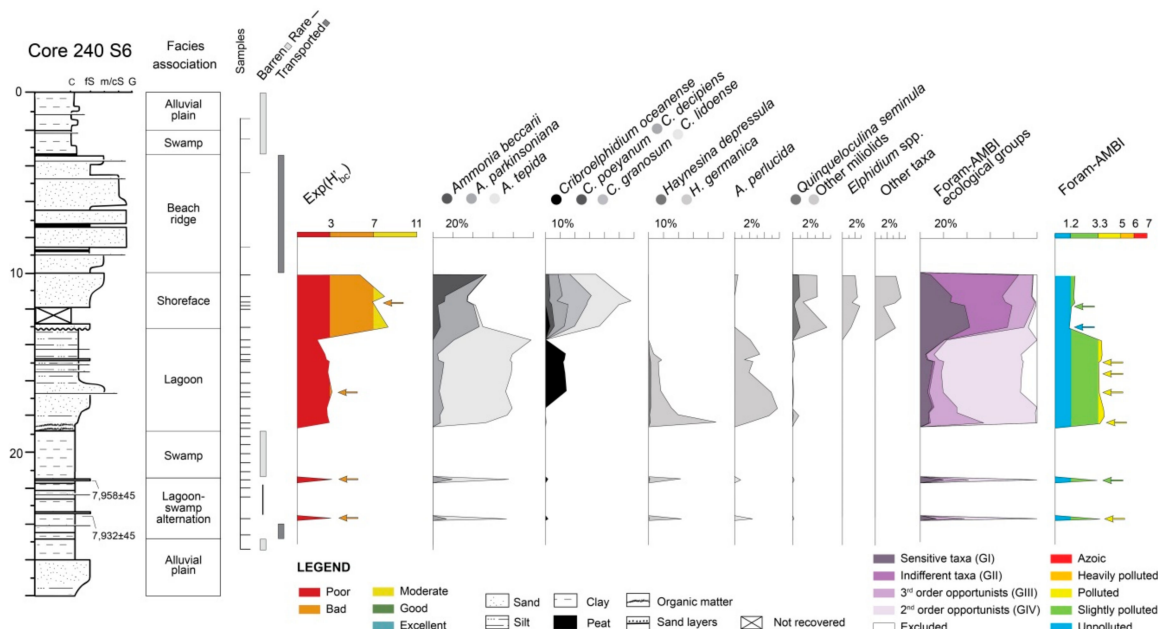


**Figure 3.** Detrended correspondence analysis (DCA) applied on the benthic foraminifer dataset of core 240 S5, showing samples and taxa colored in accordance with the Foram–AMBI ecological group. Total variance explained by each axis is reported.  $\exp(H'_{bc})$  and Foram–AMBI indices are plotted as additional variables.

#### 4.1.2. Core 240 S6

The first abundant benthic foraminifer assemblages are recorded in two single samples at 23.60 and 21.45 m core depth within the lagoon-swamp deposits, which showed  $\exp(H'_{bc})$  values of 3.1 and 3.2, respectively. Above an interval barren in benthic foraminifers attributed to a swamp paleoenvironment from 21.20 to 18.80 m core depth, lagoon sediments were mostly characterized by values of the diversity

index  $\exp(H'_{bc}) < 3$ , with a slightly higher value in one sample at 16.6 m core depth. An increase of the  $\exp(H'_{bc})$ , with values between 5.8 and 8.4, is recorded within shoreface deposits above 13.10 m core depth. PaleoEcoQs in the lowermost lagoon samples is determined as “bad”, whereas it resulted to be “poor” in the lagoon sediments between 18.80–13.35 m core depth, with the exception of one sample classified as “bad” at 16.60 m core depth. PaleoEcoQs within the uppermost coastal sediments resulted to be oscillating between “bad” and “moderate” (Figure 4, Table S1).



**Figure 4.** Stratigraphy, vertical distribution of benthic foraminifer taxa,  $\exp(H'_{bc})$ , and related PaleoEcoQs, Foram–AMBI ecological groups, and Foram–AMBI values of core 240 S6. Ages are reported as calibrated years BP. Samples barren in benthic foraminifers, including rare or transported tests are evidenced. Arrows are colored according to sample classification to improve the graphical visibility of selected samples.

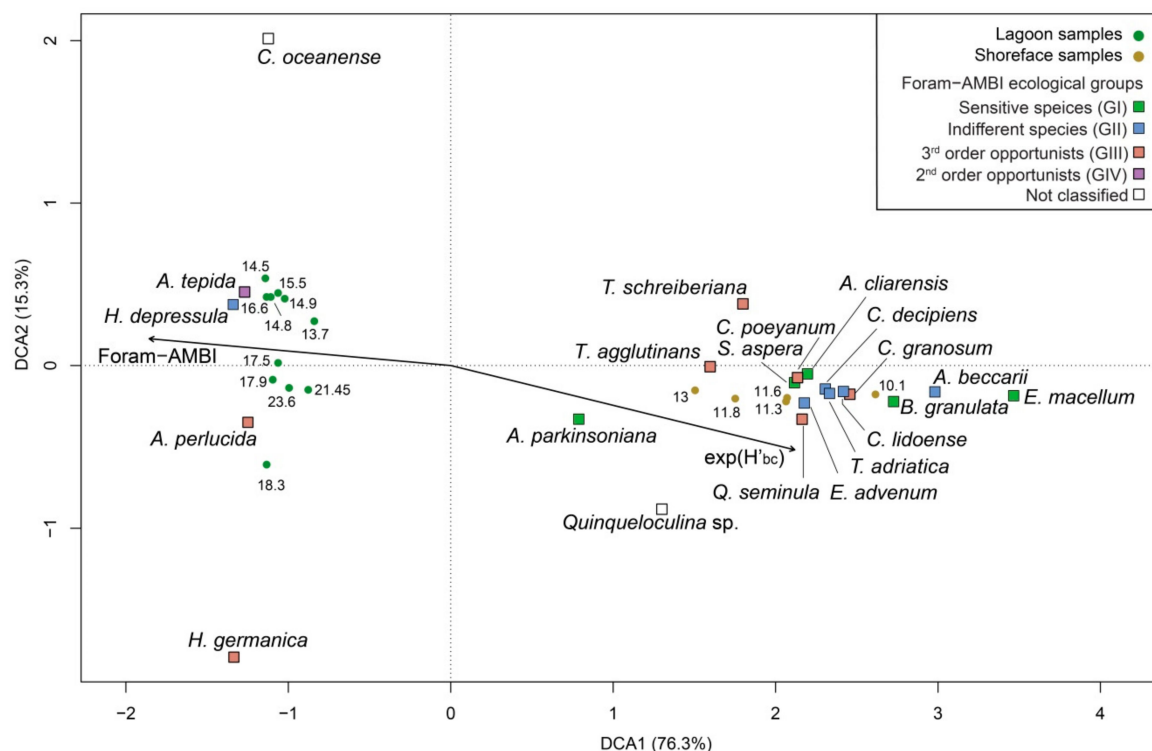
Foram–AMBI was relatively high in the lower assemblages, which showed values of 3.4 and 3.2 at 23.60 and 21.45 m core depth, respectively, and ranged from 3.3 to 3.8 up to 13.70 m core depth. Foram–AMBI dropped at 1.1 at 13.00 m core depth, and relatively low values between 1.3 and 1.5 persisted in the uppermost assemblages (Figure 4).

The low diversity and the moderately high values of Foram–AMBI in the lagoon assemblages are mostly determined by the dominance of *A. tepida* (with relative abundances even >70%), classified as second-order opportunists, which represented the most abundant taxon throughout the analyzed succession (45.7% on average). In addition, moderately high frequencies of tolerant taxa, such as *H. germanica* (with peak frequencies of ca. 45%) and *Aubignyna perlucida* (up to 5.7%; Table S1) contributed to an increase in the Foram–AMBI. Upcore, the higher diversity and the drop of Foram–AMBI within coastal sediments is mostly produced by the high abundance of indifferent species, as *Cribroelphidium lidoense* and *Ammonia beccarii* (up to 29% and 51%, respectively). These taxa are associated with a variety of sensitive species with moderate abundances, mostly represented by *Ammonia parkinsoniana* (up to >35%) and lower frequencies of *Adelosina cliarensis*, *Buccella granulata*, *Elphidium crispum*, *Elphidium macellum*, and *Siphonaperta aspera* (relative frequencies of individual taxa <5%; Table S1).

Taxa and samples in the DCA are mostly distributed along DC1 that explained 76% of the total data variance (Figure 5). Along axis one, most marine taxa sensitive or indifferent to organic enrichment are placed on the right of the plot. Whereas, the left side gathered the second-order opportunist *A. tepida* and other species, such as *H. germanica* and *Aubignyna perlucida*, tolerant to fluctuations in salinity and



organic matter and thriving in restricted, fluvial-influenced conditions [82–84]. This is confirmed by the depositional environment attributed to the samples, as negative scores characterized lagoon sediments, whereas shoreface samples showed positive scores. The orientation of environmental vectors suggests a highly positive and negative correlation of  $\text{exp}(H'_{bc})$  and Foram–AMBI, respectively, with ordination axis one.



**Figure 5.** Detrended correspondence analysis (DCA) applied on the benthic foraminifer dataset of core 240 S6, showing samples and taxa. Samples are colored according to the facies association; taxa are colored in accordance with the Foram–AMBI ecological group. Total variance explained by each axis is reported.  $\text{Exp}(H'_{bc})$  and Foram–AMBI indices are plotted as additional variables.

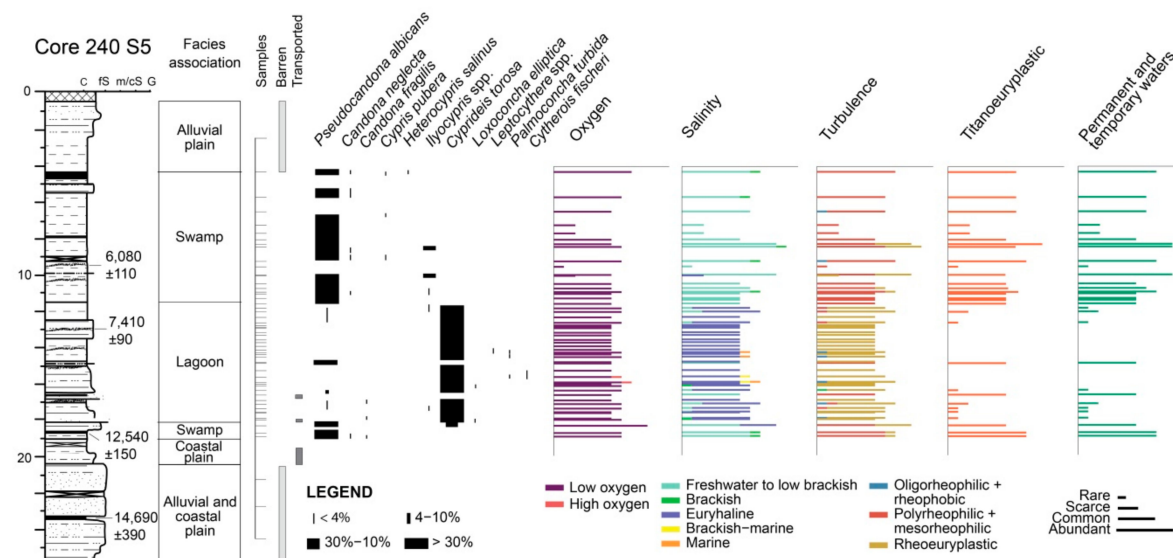
#### 4.2. Ostracods

Twenty-five ostracod taxa were identified from the two study successions. Specifically, 11 taxa were found between 18.95 and 4.30 m core depth in core 240 S5, whereas core 240 S6 included 21 taxa from 24.50 to 11.60 m core depth (Table S2).

##### 4.2.1. Core 240 S5

Ostracod taxa tolerant to low oxygen concentrations are abundant throughout the core, mostly represented by *Pseudocandona albicans*, *Candona neglecta*, and *Ilyocypris* spp. within swamp sediments, whereas *Cyprideis torosa* is dominant within lagoon sediments (Figure 6). Scattered valves of *Cytherois fischeri* recorded from 16.00 to 15.50 m core depth determined the rare occurrence of taxa characteristic of highly oxygenated conditions. According to salinity, freshwater to low brackish taxa are most abundant within swamp sediments, whereas the euryhaline *C. torosa* is dominant within lagoon deposits, that also included variable amounts of taxa tolerant to hypohaline conditions (mostly *P. albicans* and *Ilyocypris* spp.) in the lower and upper portions. Rare brackish-marine and marine species are recorded in scattered lagoon samples between 16.00 and 14.20 m core depth, represented by *C. fischeri* and *Palmoconcha turbida*. Concerning water turbulence, most taxa within swamp sediments are polyrheophilic and mesorehoophilic, and rheouryplastic within lagoon deposits, respectively,

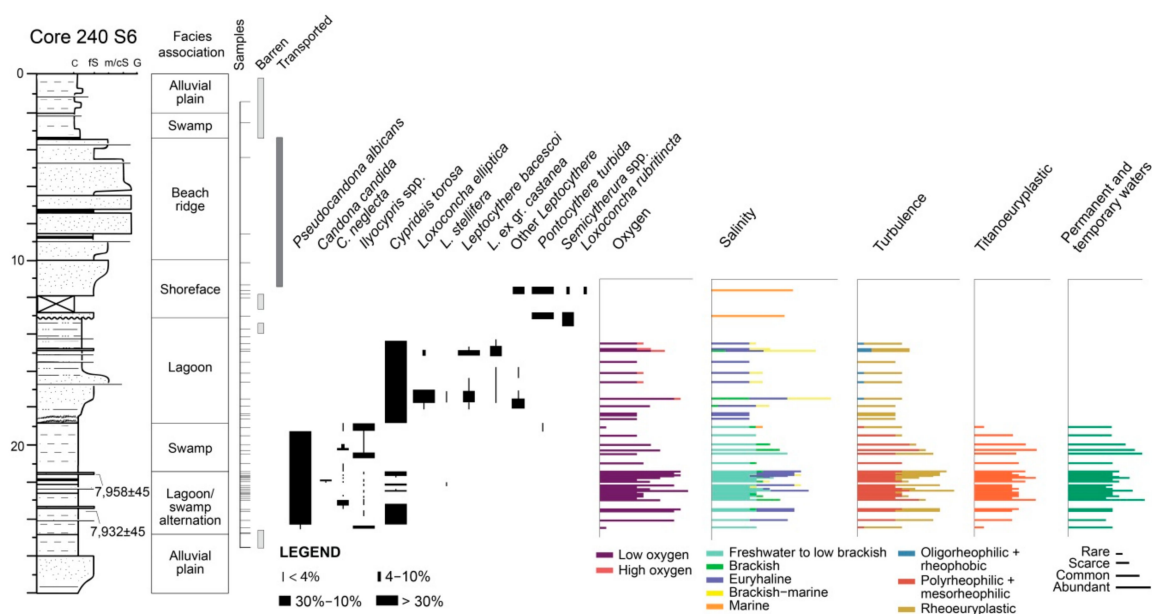
driven by *P. albicans* and *C. torosa*. Within swamp sediments, most taxa are titanoeuryplastic and able to live in both permanent and temporary waters.



**Figure 6.** Vertical distribution patterns of benthic ostracods in core 240 S5. Relative abundances of taxa and ecological groups, reported as abundance categories are shown. Samples with no or transported ostracod valves are evidenced.

#### 4.2.2. Core 240 S6

The high abundance of ostracods tolerant to reduced oxygen concentrations throughout the succession of core 240 S6 is mostly determined by the high abundance of *P. albicans* and *C. torosa* in swamp and lagoon sediments, respectively. The presence of taxa associated with high oxygen concentrations is mostly recorded in lagoon deposits from 17.70 to 13.75 m core depth due to the common to scarce occurrence of *Leptocythere ex gr. castanea*. Freshwater to low brackish species are mostly recorded in the lower part of the analyzed succession, from 24.50 to 18.80 m core depth, where the fauna is predominantly composed of *P. albicans* associated with lower occurrences of *Candona candida*, and *Ilyocypris* spp. This interval also included rare brackish and brackish-marine taxa, and very rare occurrences of the marine *Pontocythere turbida*, possibly transported from nearby marine settings. The euryhaline *C. torosa* is abundantly found within scattered intervals from 24.30 to 21.20 m core depth. Lagoon sediments from 18.80 to 13.10 m core depth include associations of euryhaline, brackish, and brackish-marine taxa (e.g., *C. torosa*, *Loxoconcha elliptica*, *Leptocythere bacescoi*), whereas exclusively marine species occur in shoreface sands. Freshwater to brackish ostracod communities are mostly composed of polyrheophilic to mesorheophilic and rheoeuryplastic taxa, whereas rheophobic to oligorheophilic species (*Leptocythere ex gr. castanea*, *L. elliptica*) occurred above 17.70 m core depth. Up to 18.80 m core depth, assemblages are composed of titanoeuryplastic taxa, characteristic of both permanent and temporary waters (Figure 7).



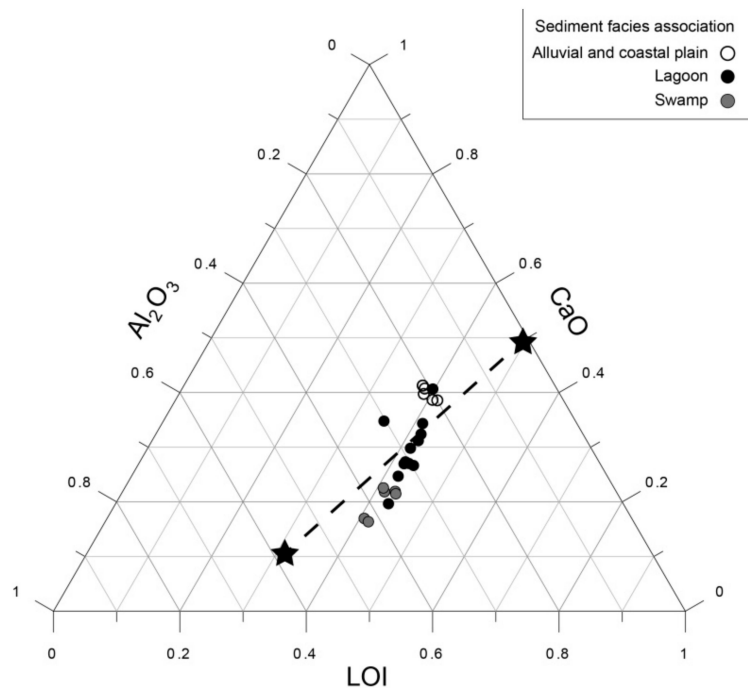
**Figure 7.** Vertical distribution patterns of benthic ostracods in core 240 S5. Relative abundances of taxa and ecological groups, reported as abundances categories, are shown. Samples with no or transported ostracod valves are evidenced.

## 5. Discussion

### 5.1. PaleoEcoQs through Short Paleoenvironmental Gradients: Restricted Back-Barrier Settings (Core 240 S5)

A relatively short paleoenvironmental gradient is depicted by the DCA samples of core 240 S5, deposited in a back-barrier, restricted lagoon setting (Figures 2 and 3). The composition of benthic foraminifer assemblages is rather stable with dominant *A. tepida* and subordinate *H. germanica*, taxa characteristic of partially barred environments enriched in organic matter and with reduced salinity [85,86]. Specifically, the former is able to rapidly consume high rates of both fresh and refractory organic matter, whereas the latter tolerates moderate organic enrichments, showing lower consumption rates of organic material [87]. The upward increasing concentrations of taxa commonly reported from lagoons but with a relatively low tolerance to organic enrichment, such as *H. depressula* and *A. parkinsoniana* [86], suggests lower organic matter levels. Despite the relatively short paleoenvironmental gradient, the Foram-AMBI index confirms the organic matter trend derived from benthic foraminifers underlain by DCA axis one (Figure 3), even under restricted conditions.

Dominant ostracod taxa (e.g., *P. albicans*, *C. torosa*) confirm abundant organic matter and reduced oxygenation, in addition to oscillating, reduced salinities and possible water turbulence during the development of lagoon conditions (Figure 6). Indeed, the dominant *C. torosa* is one of the most opportunistic ostracod species, able to thrive on extremely variable conditions, especially under high organic matter concentrations [88,89]. The scattered freshwater to low brackish ostracods within lagoon deposits, such as *P. albicans* and *C. fragilis*, also tolerate fluctuating pH and water table [76] (Figure 6) and likely suggest the development of unstable paleoenvironmental conditions. High amounts of organic materials are also suggested by geochemical indicators [25] based on the ratio between loss of ignition (LOI) and CaO of carbonates (Figure 8). LOI quantifies the total volatile content in a sediment sample, including humidity, H<sub>2</sub>O in the lattice of clay minerals, CO<sub>2</sub> in the carbonate minerals, and organic matter. Even though the individual contribution of each element cannot be discerned, the integration with selected major elements still provides a relevant assessment of partitioning among them. The majority of lagoon and swamp samples are located below the LOI-carbonate mixing line towards the LOI apex, suggesting organic enrichment and low carbonate concentrations, and possibly lowered amounts of oxygen and oscillating pH in relationship with organic matter consumption [90].



**Figure 8.** Ternary plot of CaO, Al<sub>2</sub>O<sub>3</sub>, and LOI in accordance with [36] used to assess organic matter enrichment from core 240 S5 [25]. Points located below the average pelite (lower star)–carbonate (upper star) composition line suggest high organic matter content.

Unstable paleoenvironmental conditions, confirmed by ostracod assemblages, determined the low diversity of the benthic foraminifer fauna within lagoon sediments of core 240 S5 (Figure 2). Despite the low geochemical background levels indicative of unimpacted, natural conditions, very low values of  $\exp(H'_{bc})$  resulted in the definition of “poor” or “bad” PaleoEcoQs, on the basis of threshold values defined by [21] for Italian transitional waters. This is in accordance with the Estuarine Quality Paradox, which prevents discrimination between natural and anthropogenic stresses [5,6] relying on biotic indicators, including the macrofauna [2,91].

### 5.2. PaleoEcoQs through Long Paleoenvironmental Gradients: From Transitional to Coastal Settings (Core 240 S6)

A well-developed paleoenvironmental gradient is evidenced by the DCA in core 240 S6, spanning from the lagoon to shoreface conditions (Figure 5). Species are distributed along axis one in accordance with their response to organic enrichment, which in turn is linked to the distribution of taxa in the back-barrier setting. The opportunists *A. tepida*, *A. perlucida*, and *H. germanica* are abundant in Mediterranean transitional waters, from inner lagoon to shallow prodelta settings in proximity to river outlets [22,82,86,92]. *Criboelphidium oceanense* is also reported from inner lagoon environments enriched in organic matter, and *H. depressula* seems to tolerate variable inputs of organic material in back-barrier settings, where it is commonly recorded with moderate frequencies [86,93]. The remaining taxa, mostly restricted to shoreface sediments, show a trend of increasing sensitivity to organic matter towards positive values of DCA axis one, with *A. parkinsoniana* located in an intermediate position due to its common occurrence in both outer lagoon and shallow marine settings [86,94,95]. In addition, vector orientation of the Foram–AMBI index (Figure 5) confirms a strong influence of organic matter on the benthic foraminifer distribution from back-barrier to coastal settings, which is an important factor controlling the degree of confinement in paralic environments, along with salinity, water circulation, turbidity, etc. [83].

Ostracod ecological groups confirm stressful conditions with relatively low oxygen concentration, fluctuating salinity, and water turbulence throughout back-barrier sediments (Figure 7). Above mid-Holocene lagoon-swamp alternations recording strong paleoenvironmental fluctuations

(in terms of oxygen, salinity, pH, and water table; Figure 7), thick lagoon deposits with low to moderate proportions of *Leptocythere* ex gr. *castanea* possibly suggests a tendency towards more stable, higher oxygenated conditions [78]. Nevertheless, the abundance of brackish taxa tolerant to organic enrichments such as *C. torosa*, *L. elliptica*, *L. stellifera*, and *L. bacescoi* [76,96,97] are indicative of high organic matter concentrations at the bottom and, concurrently, of increasing marine influence.

The taxonomic composition of benthic assemblages suggests a lower enrichment in organic matter and, in turn, higher oxygen conditions in core 240 S6, where  $\exp(H'_{bc})$  values are higher than in core 240 S5 (Figures 2 and 4). Similarly, the general upward increase of the diversity benthic foraminifer index  $\exp(H'_{bc})$  reflects a decrease in organic matter and the development of less restricted conditions in response to the Holocene transgression. Both back-barrier and coastal deposits are affected by an estimated PaleoEcoQs lower than it really is, which suggests a re-evaluation of thresholds values also in low confined (paleo-)environments.

### 5.3. Temporal Resolution of the Fossil Record

Living assemblages, used to define present-day EcoQs, inevitably differ from dead assemblages in taxonomic composition and structure as multiple generations are accumulated over time and space within the fossil record: this phenomenon, defined as time-averaging, has a key importance in paleoecology and, in turn, in conservation paleobiology [98,99]. In addition, taphonomic action occurring within the surface mixed-sediment layers further dampen the biological signal recorded by benthic communities [100], increasing the temporal scale of single assemblages to the decadal, centennial, or millennial time-scales [101]. Time-averaging tends to increase the diversity of assemblages, with a higher proportion of rare stenotopic taxa at the expense of abundant species [98]. Bioturbation is reported within back-barrier sediments of the Po coastal plain [51] but the presented study successions still exhibit extremely low diversity values (Figures 2 and 4), despite the potential time-averaging effects that could have mixed communities with different composition. Paralic environments were affected by rapid sediment accumulation rates, due to the high accommodation space available after the Holocene transgression (<7.0 ky BP) [57] and the high subsidence rates reached in specific depocenters, such as our study area, connected to the structural setting of the Po basin [51]. These characteristics make the transitional succession of the Po coastal plain appropriate for paleobiological investigations, especially through benthic foraminifers which are affected by a lower time-averaging than the bigger mollusk shells [102].

### 5.4. How to Define Reference Conditions?

Directions of the European Commission [4] affirmed “an urgent need” of data that would allow for the defining of reference conditions in naturally variable settings, such as transitional and coastal environments. Among benthic taxa, foraminifers resulted to be suitable biological quality elements in modern [21] and fossil [69] settings. A conservation paleobiology perspective extended through the Holocene age would cover many aspects of the natural variability of coastal systems in time and space and would also allow to investigate extended (paleo-)environmental gradients that are difficultly encountered in short-time intervals [103], ensuring an accurate assessment of PaleoEcoQs.

Geochemical data from our study material are considered as reference background values for the southern Po coastal plain [36] representing natural conditions at “high” PaleoEcoQs, nevertheless, the low-diversity associated fauna indicates low PaleoEcoQs. In the presence of anthropogenically unimpacted environmental conditions, a possible solution to overcome the Estuarine Quality Paradox issue is represented by the definition of threshold values for (Paleo-)EcoQs at a local scale, as also evidenced in pre-industrial to present-day sediment successions of the Norwegian coasts [50,69]. The principle of a location-specific approach is widely adopted in paleoecology, as dissimilarities between modern and fossil assemblages highly impact on final results (e.g., [104]). Such a location-specific approach traditionally implies an adequate sampling of modern data to develop quantitative paleoenvironmental reconstructions. In the field of conservation paleobiology and, in turn,



for biomonitoring purposes, the problem is reversed: a high-resolution chronostratigraphic sampling at a local scale would allow to cover a wide range of past analogs (i.e., reference conditions) to properly assess present-day EcoQs. In our case,  $\exp(H'_{bc})$  thresholds defined for Italian transitional waters are too high to correctly assess reference conditions in the analyzed transitional successions, despite some modern sites being located only ca. 100 km away from our study cores (e.g., Venice Lagoon; [21]). In addition, the high lateral and cross variability of transitional settings determined significant differences in  $\exp(H'_{bc})$  values, low for the lagoon assemblages of core 240 S6, and even lower in the more restricted, organic enriched setting of core 240 S5 (Figures 2 and 4) in spite of the few km among them. Taking into account such aspects, biotic indices should be lowered in accordance with faunal diversity at a very local scale.

Assemblages at high ecological status should, theoretically, decrease their diversity in the case of anthropogenic impact. Nevertheless, the assessment of EcoQs could be challenging when low diversity assemblages are already recorded under pristine conditions, as for core 240 S5. In such a case, would the first stages of anthropogenic impacts be detected? Low diversity assemblages impacted by additional stressors either locally disappear when environmental conditions overcome organisms' adaptive means or do not show changes in community structure due to the eurytopic nature of the dominant opportunistic taxa [69,105]. In such cases, diversity indices do not work properly and inferences about ecological status can be performed through geochemical indicators [69]. Indeed, further studies linking the early to mid-Holocene unimpacted assemblages with benthic communities subject to the increasing anthropogenic pressures of the last centuries are required for the Mediterranean area, in order to test benthic foraminifers as biological quality elements even in transitional environments.

## 6. Conclusions

The anthropogenically undisturbed Holocene succession of the Po coastal plain allows for the assessment of ecological conditions in transitional settings by means of benthic foraminifers, which represent biological quality elements of growing importance [13,15]. Additional paleoenvironmental insights were efficiently provided by benthic ostracods ecological groups, demonstrating excellent integration with the benthic foraminifer record.

At the locations of core 240 S5 and 240 S6, we showed that reference conditions estimated from the benthic foraminifer  $\exp(H'_{bc})$  are much lower than the "high" PaleoEcoQs expected in the pristine conditions of the Holocene sediments. In particular, the diversity decreased from coastal towards restricted back-barrier settings in accordance with the Estuarine Quality Paradox.

Within Holocene deposits naturally enriched in organic matter, where also oxygen deficiency potentially occurred, threshold values of (Paleo-)EcoQs should be established at a local scale. Values determined for Italian transitional waters underestimated the PaleoEcoQs at our study sites, which also showed significant differences among them despite the short geographical distance, due to the influence of sea-level rise.

We demonstrated that the conservation paleobiology approach offers a great opportunity to explore high ranges of natural variability throughout both short and long (paleo-)environmental gradients, in accordance with the suggestions of the European WFD [4]. The throwback to early or mid-Holocene provides "long-term" changes not necessarily recorded within recent sediment successions traditionally adopted for paleoecological purposes (i.e., back to the pre-industrial era), but possibly developed in present-day environments subject to the increasing pressure of global change.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2073-4441/12/12/3420/s1>, Text S1: List of references adopted for taxonomic attribution of benthic foraminifers and ostracods. Table S1: Benthic foraminifer abundance, Shannon H, Chao, and Shen (2003)  $H'_{bc}$ ,  $\exp(H'_{bc})$  values with related PaleoEcoQs, Foraminifera-AMBI values with related classification and percentages of individual ecological groups in cores 240 S5 and 240 S6. Table S2: Ostracod semi-quantitative abundance in cores 240 S5 and 240 S6. Table S3: Autoecological characteristics of ostracod taxa from cores 240 S5 and 240 S6, based on [76,78,79,106–109] and references therein.

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