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Article

Ostracod Fauna: Eyewitness to Fifty Years of Anthropic Impact in the Gulf of Trieste. A Potential Key to the Future Evolution of Urban Ecosystems

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Abstract: For the first time the distribution and modifications of living ostracod associations present in the Gulf of Trieste (GoT) in relation to the alterations caused by human activity in the last 20 years were investigated. The results were compared with the main chemico-physical parameters (especially nitrogen and phosphorus) measured over the same period, which can lead to a general decrease in environmental quality. For a more in-depth analysis of the changes recorded by ostracods in the last 50 years, a period in which eutrophication and anoxia increased, we revisited the study carried out by Masoli in the GoT in 1967. The results obtained made it possible to verify how over the last 20 years, ostracod assemblages have suffered a decrease both qualitatively and quantitatively. Most of the species recovered show characteristics of opportunism and tolerance to environmentally stressful conditions, high organic matter concentrations and oxygen deficiency. The ostracods analyzed in 1967 showed similar results with few dominant opportunistic species. We verified how ostracods recorded in GoT, similar to Mollusks and Foraminifera, the possible environmental crisis linked to the recurrence of mucilage and hypoxic events documented for the Gulf of Trieste in the last 50 years. Finally, a comparison with the best environmental conditions found in the Marine Nature Reserve of Miramare (MPA) allowed us to emphasize the important role of protected areas to avoid the loss of biodiversity due to urbanization.

Keywords: Urbanization; Gulf of Trieste; Ostracods; Nutrients; Environmental stress; Marine Nature Reserve.

1. Introduction

Urbanization is one of the main causes of species extinction [1]. The expansion and growth of anthropic activities decrease biological diversity because as the same ‘‘urban-adaptable’’ species become widespread and locally abundant across several ecosystems [2,3]. Thus, a great deal of research has been focused on the human influence generating ecological degradation from coastal urbanization to pollution and eutrophication [4,5]; these fundamental changes have taken place with the evolution of freshwater systems and fluxes during the last century, with particular impact seen over the last 50 years [5,6]. More specifically, drastically increased urbanization is currently one of the main sources of anthropogenic impact [7,8], often exerting significant pressure on coastal ecological systems [9].

The need to start efficient mitigation activities to assess and manage the negative impacts of urbanization on natural habitats (i.e., to reduce the urban footprint and preserve habitats in urbanized areas) have highlighted the importance of investigating anthropic impact and the response

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of contiguous marine ecosystems in recent times. In this context GoT represents a suitable area for investigating recent human impact on a coastal marine environment, as this area has been densely urbanized over a long period and subject to agricultural activities in the inland Friulian Plain, the presence of wastewater discharge and the riverine inputs has caused episodes of anoxia [10] similarly to those observed in others coastal areas [5,11,12,13].

The GoT is characterized by several sources of pressures such as industrial sites, two busy harbors (Trieste and Monfalcone), intense ship traffic, aquaculture, mussel farming and tourism facilities.

The effects of anthropogenic disturbances in the GoT were investigated taking into account the presence and distribution of geochemical pollutants [14,15,16,17,18,19,20,21], biological markers [22] and by using an integrated approach [23,24]. On this basis, a restricted area of the GoT was identified as heavily polluted and defined as a Site of National Interest (SINs) (d.lgs. 22/97; d.lgs. 152/2006), which required clean-up [25].

Recent analysis investigated the relationship between the benthic communities and the anthropization in areas of growing urbanization [4,26]. [4] recorded a decline in some molluscan species in the second half of the twentieth century as well as an associated increase in some opportunistic species as a result of an increase in fishing and hypoxia phenomena. The recent studies on foraminifera communities [27,28] highlighted the response of this taxon to the various stimuli linked to anthropogenic factors and, consequently, to the degree of environmental quality, which is directly related to levels of pollutants and/or trophic state (i.e., anoxia/hypoxia phenomena).

A sensitive species, ostracods are small crustaceans (from 500 to 1500 mm in length which occupy all aquatic environments (from the deep sea to inland freshwater ponds and wetland to terrestrial environments) and are capable of secreting calcareous carapaces. Their distribution is controlled by hydrological, biological and sedimentological features. These organisms are useful for environmental characterization on a local/regional scale and can indicate water depth, salinity, temperature and other ecological factors. Numerous authors have reported the use of ostracods for environmental and paleoenvironmental studies as sentinels of anthropic impacts and associated pollution and hypoxia phenomena [29,30,31,32,33,34,35,36,37,38,39,40,41]. In particular, some studies have focused on the response of these organisms to coastal human impact in terms of water pollution by industrial and agricultural processes, sewage and the resulting eutrophication that may lead to hypoxia and, in extreme cases, may induce anoxia [42,43]. In this context, ostracods are usually intolerant of hypoxia and respond by a reduction in diversity and richness, and, in some cases, populations become monospecific [5,44,45].

In this work, we examined the ostracods population in the GoT over the last 20 years, by applying an integrated, multidisciplinary approach, to reconstruct and evaluate the recent history of the impacts of the urbanization on communities of these small crustaceans. The main chemico-physical parameters commonly employed to define the trophic state (i.e., nutrients, chlorophyll *a*, temperature, salinity) were also taken into consideration to check the relationships with changes in population. In addition, we compared the more recent data with the results published by [46] to verify changes in association (appearance and disappearance) recorded over a time span of 50 years.

The evaluation of their modifications and adaptations over time could represent a crucial factor in setting out future recovery actions and to implement a sustainable urban plan, often invoked as a ‘win–win–win’ scenario to optimize economic, environmental and social goals [47].

2. Materials and Methods

2.1 Study area

The GoT is located in the northernmost part of the Adriatic Sea (Italy). It is an epicontinental semi-enclosed shelf basin covering an area of approximately 500 km^2 with a maximum water depth of 38 m (25 m average depth 10 m) and characterized by a very low bathymetric gradient. It is affected by the significant contribution of continental waters coming from the Italian and Istrian regions [48].

The primary freshwater input is represented by the Isonzo/Soča River (annual flow rate of 82 m^3 s^{-1}) and the contribution of several minor rivers (Timavo/Reka, Rosandra/Glinščica, Ospo/Osp, Rižana, Badaševica, Drnica and Dragonja) can be considered negligible or having only local effects. Water circulation is driven by the interplay of various forcing factors: the general circulation of the Adriatic Sea, winds (particularly the dominant Bora, N-NE direction), buoyancy fluxes together with tides. The GoT represents a site of shelf dense water formation that contributes to the North Adriatic Deep Water.

The sediments texture varies from medium to fine sands along the coastline and the delta of the Isonzo and Tagliamento Rivers, to muds in the mid-Gulf and sandy sediments in the western open part of the GoT. Carbonate sediments dominate the sediments near the river' mouth [49,50].

The GoT is a suitable site to study anthropic impact since, in spite of its relatively small extension, it hosts two of the largest cargo shipping ports in the Adriatic Sea (Trieste and Koper). This coastal area is affected by many potential sources of organic and inorganic pollutants, discharged not only by rivers but also by sewers, industrial developments, and harbor related activities including an oil-pipeline terminal [51,52]. Moreover, the site has been recognized as an area where particular conditions related to inputs of fluvial sediments or to meteo-marine conditions led to significant algal productions and blooms, resulting in eutrophication and the subsequent hypoxic/anoxic conditions at the bottom, at least until the mid-1980s [53].

2.2 Experimental Site

The experimental site is located in the GoT (13°37′ E to 13°44′ E and 45°41′ N to 45°44′ N). Within this area 44 samples were collected during two different summer cruises conducted in 2004-2005, and 2013-2017, hereafter referred to as GTCrB and GTCrC, respectively (Figure 1). Sampling was performed using a five liters Van Veen grab and a KC Haps bottom corer characterized by a sample area of 0.013 m^2 with an effective depth penetration of 10 cm.

In order to assess the changes which have occurred in the GoT since 1967, we applied the GIS methods to reconstruct the exact georeferenced location of the samples collected by the "Istituto di Geologia e Paleontologia" (University of Trieste) during two summer cruises conducted in 1965 and 1966, respectively (site GTCrA) and compared the qualitative analyses of ostracods reported by [46] with those ostracods recovered in 2004 and 2017 (Figure 1).

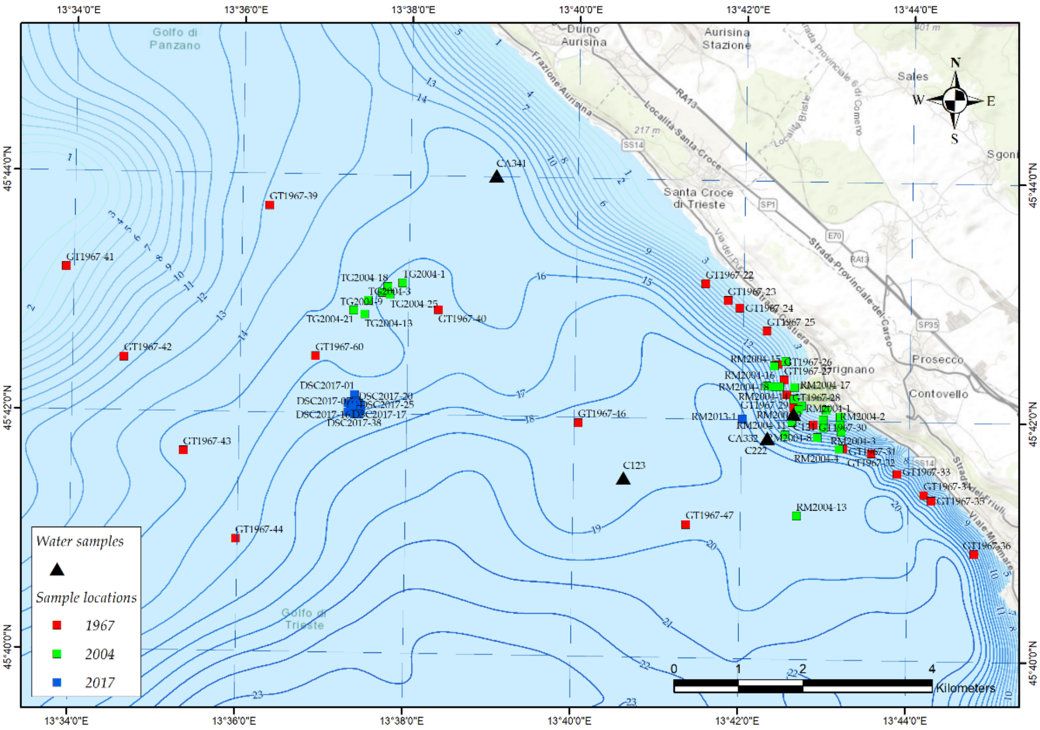

Figure 1. Location of the study area and sampling stations.

The collected sediments were wet-sieved using a 63µm mesh, dried and weighed to determine the content of the sandy fraction. The total sandy sediments were used to collect all live benthic specimens, representative of the environmental conditions, and to avoid problems related to the potential presence of the reworked fauna originating from deposits created in connection with the Early Holocene marine transgressions and the Holocene climatic optimum as indicated by [54] in the eastern part of the northern Adriatic Sea. Ostracod analysis took into account monographs and papers from the Mediterranean literature [55,56,57]. Particular attention was paid to the papers concerning the northernmost sector of the Adriatic Sea corresponding to the GoT [46,58,59].

To characterize the biodiversity of assemblages, two faunal parameters were calculated: (1) species diversity (S), the number of species in each sample; (2) the Shannon–Weaver index (H), a measure of entropy that takes into considerations the distribution of taxa among the total individuals [60] (Table 1).

Multivariate analysis on the GTCrB and GTCrC ostracod assemblages was performed using the Xlstat software Addinsoft (2020) (XLSTAT statistical and data analysis solution. New York, USA. https://www.xlstat.com), except for the calculation of SIMPER and of diversity indices performed using PAST software (PAlaeontological STatistic – version 4.02) [61]. Cluster analysis was run for samples (Q mode). The best results were reached using Ward's method and the Bray and Curtis algorithm. Also included was the presence/absence data used in this analysis for comparative purposes and both provided the same results, thus confirming the validity of the obtained groups.

Table 1. Diversity indices (species diversity, Shannon–Weaver) calculated for benthic living ostracods in each sample.

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Samples	**Taxa_S**	**Individuals**	**Shannon_H**
TG2004-1	13	41	2.4
TG2004-3	8	16	2.0
TG2004-13	19	53	2.7
TG2004-18	14	57	2.2
TG2004-21	14	110	1.9
TG2004-25	14	56	2.2
RM2004-1	8	16	1.6
RM2004-2	9	45	1.5
RM2004-3	11	76	2.1
RM2004-4	11	84	2.0
RM2004-5	5	6	1.7
RM2004-6	14	69	1.6
RM2004-7	6	44	1.2
RM2004-8	6	38	1.5
RM2004-9	14	64	1.9
RM2004-10	13	96	1.8
RM2004-11	11	99	1.9
RM2004-12	10	108	1.9
RM2004-13	8	26	1.8
RM2004-14	7	55	1.7
RM2004-15	6	19	1.5
RM2004-16	11	77	2.0
RM2004-17	5	21	1.4
RM2004-18	10	76	1.3
RM2004-19	9	68	1.3
RM2004-20	10	93	1.8
DSC2017-01	6	9	1.7
DSC2017-07	5	41	1.0
DSC2017-10	7	8	1.9
DSC2017-12	7	61	1.2
DSC2017-13	8	31	1.3
DSC2017-16	7	26	1.3
DSC2017-17	5	13	1.4
DSC2017-18	3	9	0.7
DSC2017-19	4	15	0.7
DSC2017-20	4	7	1.3
DSC2017-21	3	4	1.0

DSC2017-25	8	24	1.7
DSC2017-32	4	4	1.4
DSC2017-34	6	18	1.4
DSC2017-38	1	1	0.0
DSC2017-39	1	1	0.0
RM2013-1	13	58	2.0

2.3 GIS analysis

Predictive distribution maps for critical species and the Shannon Index have been interpolated in GIS using the Inverse Distance weighting method (IDW). The measured relative frequency values surrounding the prediction location have been used to predict a value for any unsampled location in the study area, based on the assumption that things that are close to one another are more alike than those that are farther apart. IDW is a weighted distance average and so the predicted value is limited to the range of the values used in the interpolation.

Unlike other interpolation methods—such as Kriging,—IDW does not make explicit assumptions about the statistical properties of the input data. IDW is often used when the input data do not meet the statistical assumptions of more advanced interpolation methods.

IDW assumes that each measured point has a local influence that diminishes with distance. It gives greater weight to points closest to the prediction location, and the weight decreases as a function of distance raised to a power value (p=2 in our case). The search neighborhood can be altered by changing its size and shape and/or by changing the number of neighbors included. The maximum and minimum number of neighbor measures to include has been set and the neighborhood search has been divided into sectors to account for any directional autocorrelation or trend in the data [62].

2.4 Nutrient analyses and multiprobe data acquisition

For nutrient analyses surface water samples were collected with a horizontal Niskin bottle (V = 5 l). For the determination of dissolved nutrients (ammonia, $N–NH_4^+$, nitrite, $N–NO_2^-$, nitrate, $N–NO_3^-$, soluble reactive silicate, SRSi, and soluble reactive phosphorus, SRP) samples were collected in HCl acid-washed polyethylene bottles (V = 100 ml) after filtration by means of GF/F fiber filters (Millipore, 0.45 µm), and immediately frozen (T = −20 °C) until analysis. The nutrients were always determined by means of the segmented flow technique (Bran + Luebbe AutoAnalyzer 3 and QuAttro) following the methods reported in [63] and modified for the specific instrument. The specific calculated method detection limits were 0.02 µM for $N–NH_4^+$, $N–NO_2^-$, $N–NO_3^-$, and 0.01 µM for SRP and SRSi. Certified standards (Inorganic Ventures Standard Solutions and MOOS-2, NRC) were used to ensure the accuracy of the procedures. In addition, the analytical performance was periodically checked through proficiency tests (PT) exercises organized by the European network of PT providers (QUASIMEME programmes AQ1 and AQ2).

During sampling, water column vertical profiles of pressure (dbar), temperature (°C), conductivity (mS/cm), salinity, pH, dissolved oxygen (% saturation and mg l^{-1}) and chlorophyll *a* (as an estimate of phytoplankton biomass, µg l^{-1}) from the surface to the bottom were collected on board

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using an Idronaut mod. 316 (2004-05; 2013) and an Idronaut mod. 316 plus (2014-17) multiparametric probes, which were calibrated following the manufacturer's protocols. The data obtained were processed using Idronaut software in order to verify the quality check.

All the chemico-physical data were processed in order to determine means, median, standard deviations, and maximum and minimum values, and were graphically displayed as boxplots using the free PAST software version 2.06. Spearman correlation coefficients (r) indicate the strength and direction of a linear relationship between variables; r was considered significant when the p-value was < 0.05. The trophic state was calculated by applying the TRIX index [64]. This index combines nutrients (DIN as sum of $N–NH_4^+$, $N–NO_2^-$, $N–NO_3^-$ and TP expressed as $\mu g\ l^{-1}$ of N and P, respectively), Chl *a* ($\mu g\ l^{-1}$) and DO (absolute deviation from % saturation).

3. Results

3.1 Ostracods evolution

52 species were identified in the examined area: 37 and 24 species in GTCrB and GTCrC respectively, while [46] found 25 species in the same area (Table S1). Dominant species in the GTCrB samples are *Aurila convexa*, *Carinocythereis whitei*, *Cytheridea neapolitana*, *Leptocythere ramosa*, *Loxoconcha ovulata*, *Pseudopsammocythere similis*, *Pterygocythereis jonesii*, *Semicytherura incongruens*, while in GTCrC they are outnumbered by *A. convexa*, *L. ovulata*, *Loxoconcha rhomboidea*, *S. incongruens*, *Xestoleberis communis* and *Xestoleberis dispar* (Plate A1).

In the same area, [46,58] recorded the prevailing presence of *Callistocythere adriatica*, *Callistocythere flavidofusca*, *Carinocythereis carinata*, *Cushmanidea elongata*, *C. neapolitana*, *Loxoconcha tumida*, *Palmoconcha turbida*, *P. jonesii*, *S. incongruens*, *X. communis* and *X. dispar* (Table S1).

The H index ranges between 2.7 (sample TG2004-13) and 0 (samples DSC2017-38 and DSC2017-39). Higher values were recorded in samples from GTCrB with an evident drop in values in GTCrC (Table 1).

The comparison between the different analyzed periods showed continuity over time in the examined area of the following species: *A. convexa*, *C. adriatica*, *C. neapolitana*, *P. jonesii*, *S. incongruens*, *X. communis* and *X. dispar.*

The species *Callistocythere flavidofusca, Cushmanidea elongata, Cytheretta subradiosa, Hiltermannicythere turbida, Leptocythere multipunctata, Loxoconcha avellana, Loxoconcha tumida* and *Schedopontocypris setosa* were found only in GTCrA.

The Q-mode cluster analysis performed on the ostracod associations found in GTCrB and GTCrC reveals the presence of three groups of samples (Figure 2).

The SIMPER analysis shows that more than 60% of the difference between clusters is defined by *S. incongruens* (24.4% relative contribution), *A. convexa* (9.9 %), *C. neapolitana* (9.9 %), *L. ovulata* (8.6 %) and *X. communis* (8.3 %) followed by lower contributions of *Leptocythere ramos*a (6.8 %), *P. jonesii* (3.8 %) and *Carinocythereis whitei* (3.5 %).

Cluster 1 is dominated by *A. convexa, L. ovulata* and *X. communis*. Relatively low numbers of *C. neapolitana, Loxoconcha affinis* and *L. rhomboidea* are recorded (specifically, within the samples DSC2017-01, RM2004-5, TG2004-21). *S. incongruens* dominate cluster 2, followed by very low frequencies of *C. neapolitana* and *C. whitei* recorded in scattered samples. Although cluster 3 is still dominated by *S. incongruens*, it includes high concentrations of several other species, such as *C. neapolitana*, *C. whitei*, *L. ramosa* and *P. jonesii* (Table 2).

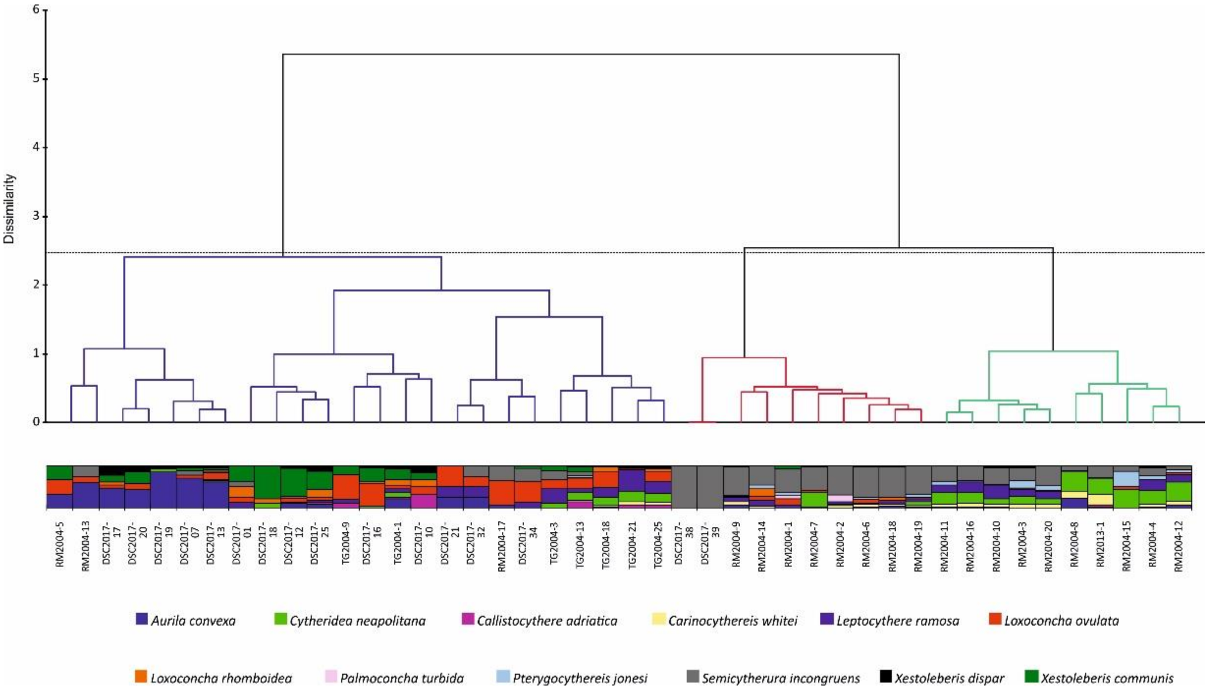

Figure 2. Q mode Cluster analysis (Ward's method - Bray and Curtis algorithm). The dominant species are reported.

Table 2. Similarity percentage (SIMPER) analysis for ostracod assemblages defined with Q-mode cluster analysis. Overall average dissimilarity: 79.17.

Species	Av. dissim	Contrib. %	Cumulative %	Cluster 1	Cluster 2	Cluster 3
Semicytherura incongruens	19.3	24.4	24.4	5.7	63.3	22.9
Aurila convexa	7.8	9.9	34.3	18.2	1.7	3.2
Cytheridea neapolitana	7.8	9.9	44.2	4.3	4.3	25.4
Loxoconcha ovulata	6.8	8.6	52.8	17.9	4.2	1.8
Xestoleberis communis	6.6	8.3	61.1	16.0	1.0	0.1
Leptocythere ramosa	5.4	6.8	68.0	8.4	3.6	13.0
Pterygocythereis jonesi	3.0	3.8	71.7	0.1	2.7	8.7
Carinocythereis whitei	2.8	3.5	75.3	0.7	3.1	8.6
Loxoconcha rhomboidea	2.2	2.7	78.0	4.2	2.3	0.0
Xestoleberis dispar	1.9	2.3	80.3	4.3	0.4	1.1
Pseudopsammocythere similis	1.8	2.3	82.6	1.1	2.0	4.6
Cistacythereis turbida	1.4	1.7	84.3	0.5	1.9	3.1
Leptocythere bacescoi	1.2	1.5	85.8	0.0	3.2	1.2
Cytheroma variabilis	1.2	1.5	87.3	0.1	0.3	3.8
Loxoconcha affinis	1.1	1.4	88.7	2.3	0.8	0.0
Callistocythere adriatica	1.1	1.4	90.2	2.5	0.0	0.5

Semicytherura rarecostata	1.1	1.4	91.5	2.1	1.0	0.0
Cytherois triangularis	1.0	1.2	92.7	2.3	0.0	0.0
Leptocythere fabaeformis	0.9	1.1	93.8	2.1	0.0	0.0
Propontocypris dispar	0.6	0.8	94.6	1.2	0.3	0.3
Palmoconcha turbida	0.6	0.8	95.4	0.1	2.0	0.0
Loxoconcha stellifera	0.5	0.6	96.1	0.8	0.6	0.0
Callistocythere sp.	0.4	0.6	96.6	0.1	1.1	0.3
Sclerochilus contortus contortus	0.4	0.5	97.2	1.0	0.0	0.0
Carinocythereis carinata	0.3	0.4	97.6	0.8	0.0	0.0
Palmoconcha subrugosa	0.3	0.4	97.9	0.7	0.0	0.0
Costa edwardsi	0.3	0.3	98.3	0.1	0.0	0.8
Triebelina raripila	0.2	0.3	98.6	0.4	0.3	0.1
Aurila fallax	0.2	0.3	98.8	0.5	0.0	0.0
Paradoxostoma triste	0.1	0.2	99.0	0.3	0.0	0.0
Aglaiocypris sp.	0.1	0.1	99.1	0.2	0.0	0.0
Semicytherura sulcata	0.1	0.1	99.2	0.2	0.0	0.0
Microcythere depressa	0.1	0.1	99.4	0.0	0.2	0.2
Eucythere curta	0.1	0.1	99.5	0.2	0.0	0.0
Paradoxostoma acuminatum	0.1	0.1	99.6	0.2	0.0	0.0
Semicytherura inversa	0.1	0.1	99.6	0.2	0.0	0.0
Carinocythereis antiquata	0.1	0.1	99.7	0.0	0.0	0.2
Microcythere gibba	0.1	0.1	99.8	0.0	0.0	0.2
Propontocypris pirifera	0.1	0.1	99.8	0.0	0.0	0.2
Paracytheridea depressa	0.0	0.0	99.9	0.1	0.0	0.0
Cyprideis torosa	0.0	0.0	99.9	0.1	0.0	0.0
Cytherois frequens	0.0	0.0	100.0	0.1	0.0	0.0
Pontocypris acuminata	0.0	0.0	100.0	0.1	0.0	0.0

3.2 Physico-chemical variables

Table 4 lists the descriptive univariate statistic of the physico-chemical variables considered in this study in the periods from 2004-2005 and 2013-2017. The water temperature (T) showed the typical patterns of the Mediterranean area, with minimum values recorded at the beginning of March 2005 (5.98 °C) and the maximum in June 2013 with 28.2 °C. Salinity (S) values generally depend on the degree of freshwater inputs from the Isonzo River, which are strongly related to rainfall [65]. As a result, the early spring and autumn displayed the lowest values with an outlier of 8 recorded in November 2014, likely during a period of high discharge from the river. Dissolved oxygen (DO), expressed as % of saturation, ranged from 80.4 to 129%, whereas chlorophyll *a* (Chl *a*), which is a good estimate of phytoplankton biomass, ranged from 0.1 to 2.47 μg l^{-1}, thus the occurrence of significant algal blooms can be excluded for both periods investigated. These results are comparable to those reported in a time series for the period from 1970-2007 in the whole Northern Adriatic basin [66].

N-NO_3^- was the predominant form of dissolved inorganic nitrogen (DIN). In fact, on average it accounted, for 86.5 and 77.1% of total dissolved nitrogen for the periods from 2004-2005 and 2013-2017, respectively. The lowest mean values were found in summer (0.41±0.45 μM, 2004-2005), whereas in spring and autumn the N-NO_3^- content increased due to riverine inputs. Certain European Directives give threshold values for DIN (74/440/EEC; 76/464/EC; 78/659/EC; 80/68/EC; 98/15/EC). Taking into consideration the whole data set DIN did not exceed these values (DIN<15 mg l^{-1} N-1072 μM N; N–NO_3^- <25 mg l^{-1} N-403 μM N; N–NH_4^+ <1 mg l^{-1} N-71 μM N). Finally, SRP ranged from <loq to 0.36 μM P.

The DIN: SRP molar ratio is commonly used to detect whether N and P act as factors capable of limiting primary production [67]. In this work the ratio was always higher than 16 suggesting that the system is P-limited [68].

Several criteria are commonly used to define the trophic state in aquatic systems. The TRIX index was set by [69] and according to these authors the quality varied from high, characteristic of a system with low productivity and low trophic level (TRIX: 2–4), to poor, typical of a highly productive system with high trophic levels (TRIX: 6–8). In this work, TRIX ranged from 2.19 to 4.06 (average based on seasonal aggregated data), which is consistent with a low trophic level, especially during summer periods, and good water quality.

Pearson linear correlations between variables are shown in Figure 4, where the significant correlations ($p<0.05$) are boxed. A strong negative correlation of S with oxidized nitrogen form (N-NO_3^-), SRSi, TP and TN was observed, especially during the period from 2013-2017, whereas Chl *a* contents were scarcely correlated with nutrients, thus indicating that nutrient inputs are not sufficient to cause a significant primary productivity. Nutrients are positively correlated amongst themselves suggesting their common origin.

The results obtained in the two periods, for some chemico-physical parameters (T, S, Chl *a*, N-NO_3^- and SRP), were compared in order to check if a significant increase or decrease had occurred: these factors can influence the distribution, richness and diversity of benthic organisms. For these purposes, we applied the Kruskal-Wallis test for equal medians. The results showed a significant increase in T, Chl *a* and N-NO_3^-, whereas S and SRP significantly decreased.

Table 3. Univariate statistic for chemico-physical parameters.

2004-2005	T (°C)	S	Chl *a*	O2 (%)	N-NO2	N-NH4	N-NO3	P-PO4	Si-SiO2	TN	TP
N	117	117	117	117	112	114	117	116	117	117	117
Min	5.99	28.71	82.9	0.10	0.01	0.01	0.01	0.01	0.15	5.11	0.17
Max	26.841	38.43	112.3	2.00	1.56	2.00	22.88	0.19	12.13	34.25	4.49
Mean	14.51	36.97	98.34	0.59	0.40	0.74	3.10	0.07	3.20	12.75	0.82
Stand. dev	5.80	1.62	6.09	0.45	0.45	0.44	3.92	0.04	2.30	5.66	0.63
Median	13.57	37.44	98.1	0.5	0.21	0.71	1.9	0.06	2.6	11.17	0.66
25 prcntil	9.23	36.81	93.7	0.2	0.07	0.36	0.69	0.04	1.48	9.18	0.53
75 prcntil	18.49	37.91	103.5	0.8	0.53	1.04	4.21	0.09	4.42	14.58	0.93

2013-2017	T (°C)	S	Chl *a*	O2 (%)	N-NO2	N-NH4	N-NO3	P-PO4	Si-SiO2	TN	TP
N	23	23	23	22	23	23	23	21	22	23	21

Min	11.8	9	0.1	86	0.02	0.05	0.77	0.01	0	0	0
Max	20.5	37.8	2.47	117	1.71	5.81	59	0.32	83.25	74.86	0.4
Mean	15.68	35.19	0.97	97.82	0.56	1.40	6.58	0.09	10.84	17.80	0.10
Stand. dev	2.65	6.00	0.62	7.37	0.58	1.51	12.16	0.10	17.35	16.44	0.08
Median	15.7	37.1	1.03	97.8	0.3	0.9	2.86	0.04	6.84	11	0.08
25 prcntil	13.6	36.5	0.4	93.48	0.07	0.42	1.76	0.03	2.34	9.04	0.06
75 prcntil	17.5	37.4	1.32	101	1.18	1.53	5.25	0.12	12.32	23.57	0.13

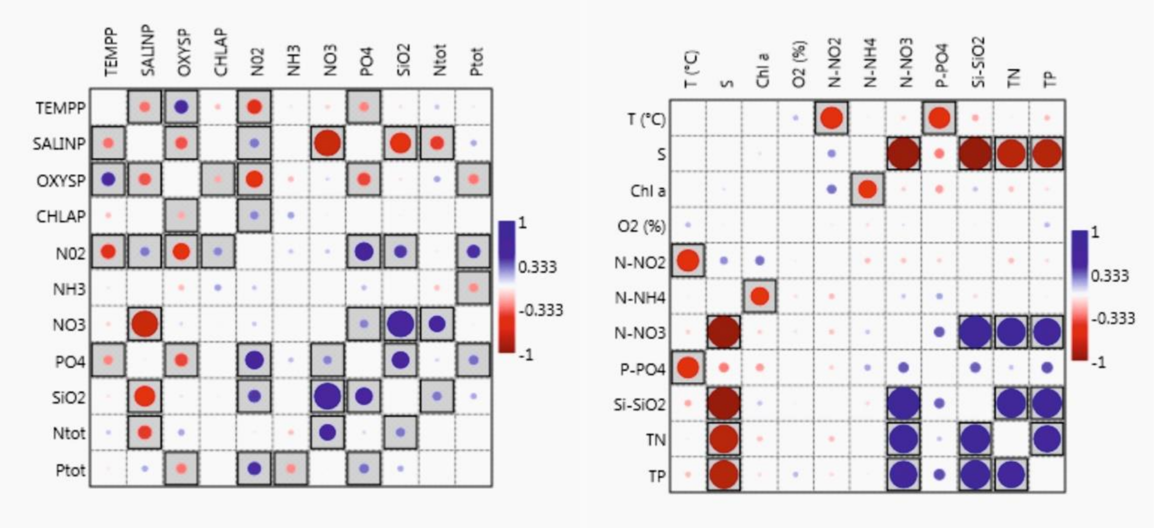

Figure 3. Pearson linear correlations between variables, significant correlations (p<0.05) are boxed.

4. Discussion

Recent numerous works have highlighted the variations and impact on ecosystems of recent and growing urbanization at a global level. Urbanization may filter out species that are not preadapted to urban conditions, with a subsequent decrease in abundance or diversity at small (local) scale [8, 70].

Alternatively, the loss of species less adapted to urban environments could be (over)compensated for by an increase in species efficient in exploiting urban resources [2,3,71]. Both phenomena may cause biotic homogenization if local communities are colonized by the same species, in turn increasing the compositional similarity of urban species assemblages and, consequently, reducing species richness of urban areas on a large scale [2, 72]. The relationship between the growth of cities and the impact of human activities on adjacent marine areas is still currently difficult to analyze. [5,43] recorded how, in marine realms, the predominant cause of degradation noticed in microfossil records was nutrient enrichment and the resulting symptoms of eutrophication including hypoxia.

In particular, in the Adriatic Sea, changes were caused by eutrophication and anoxia due to human activities including agriculture, wastewater disposal, and diversion of river outflow [12,13]. In this sense, ostracods are usually intolerant to hypoxia and respond with a reduction in diversity and richness, and, in some cases, populations become monospecific [44,45].

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In the northern Adriatic ecosystems and the GoT area, a review of numerous long term studies on river discharg, oceanographic features, plankton, fish and benthic compartments, collected since the 1970s revealed significant changes in mechanisms and trophic structures [53,73]. In detail, a gradual increase in eutrophication phenomena, characterized by significant hypoxic events at the bottom, was recorded during the 1970s until the mid-1980s [74,75,76], followed by a reversal of the trend, particularly marked in the 2000s [66,77]. This trend was attributed to a combination of the reduction in anthropogenic impact, mainly due to a substantial decrease of the phosphorus loads, and of climatic modifications, resulting in the decline in atmospheric precipitations and, consequently, of runoff [78,79]. However, the occurrence of significant atmospheric phenomena due to climate change are indicated by the increase in N-NO_3^- and the decrease of salinity: long drought periods are followed by heavy rainfall that increase the Isonzo River discharge for short periods.

The analysis of the molluscan community composition in the Bay of Panzano cores [4] recorded how frequent past hypoxic events, intensifying pressure from fishing and climatic factors can replace contamination as the main drivers of community change leading to the most pronounced shifts in molluscan community composition. In the second half of the 20th century, disturbances from fishing and hypoxia intensified with the benefit of opportunistic species. Results from the foraminiferal record in the same cores confirm eutrophication as the most significant driver of community shifts [26].

To verify the effects of urbanization on ostracod fauna over the last 50 years, the experimental site was carefully chosen at the center of the innermost part of the GoT. Moreover the presence within the area of the MPA (EUAP 0167), established in 1986 by a decree from the Italian Ministry of the Environment, which has entrusted the management to WWF Italy onlus Association (D.M. November 12, 1986) and from 2013 included in SCI - Site of Community Importance - list (directives 79/409/EEC and 92/43/EEC) (http://www.riservamarinamiramare.it), afforded the opportunity to analyze potential ostracod response in environments subject to varying degrees of environmental stress.

Sediments in the GoT are also dispersed regularly in concentric bands with respect to the mouth of the main rivers with dominant sedimentation in the experimental site mainly characterized by a sandy pelite and very sandy pelite [49]. All the samples examined are additionally included at a reduced depth range so both these parameters can be considered irrelevant in the distribution of ostracod association.

The analysis of ostracod fauna showed a clear quanti/qualitative decrease from GTCrB to GTCrC. The only exception is sample RM2013-1 collected in MPA, which shows values comparable with GTCrB associations (Table S1). The Shannon index values further confirm the above data with a clear decrease in all recent samples except for RM2013-1 (Figure 4).

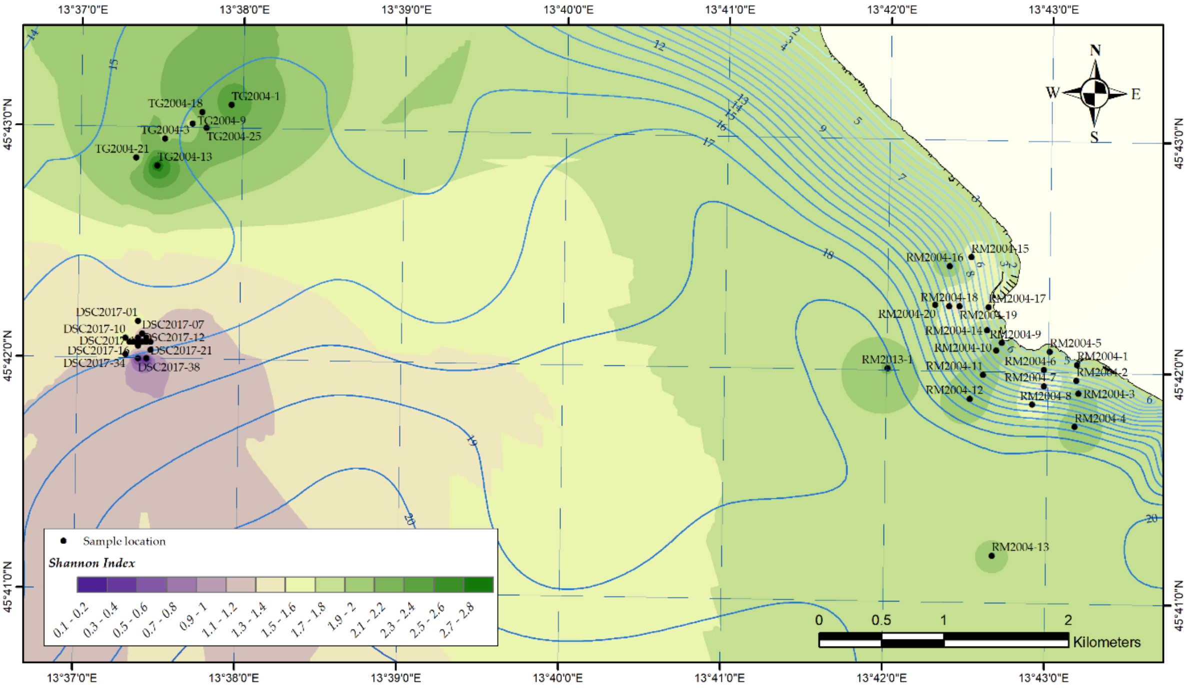

Figure 4. Shannon Index interpolated in GIS using the Inverse Distance weighting method (IDW).

The indications coming from the analyzed species over a time span of 20 years (GTCrB and GTCrC), records in both samplings a dominance of species tolerant to high organic matter concentrations and oxygen deficiency such as *C. neapolitana, L. ramosa, Loxoconcha* spp. [80]. In GTCrC samples, the trend towards homogenization is further amplified with the domain of few opportunistic species represented by *A. convexa, C. Neapolitana, Loxoconcha* spp., *S. incongruens* and *Xestoleberis* spp. (Figure 5). All these species are known to be hypoxia-tolerant or opportunistic [5,31,43,81] (Figure 5). In addition, [38] recorded the presence of living specimens of *X. communis* and *X. dispar* in stressful environmental conditions such as the Ex-Military Arsenal of the La Maddalena Harbor, both species were found to be dominant at GTCrC.

In GTCrA, even when cautiously viewed through the lense of qualitative analysis, the ostracod association from 1967 differs from those found recently. The number of species reported by [46,58] is numerically lower than GTCrB, but the association shows the presence of species no longer found in recent samples (*C. flavidofusca, C. elongata, C. subradiosa, H. turbida, L. multipunctata, L. avellana, L. tumida* and *S. setosa*) (Table S1). Among these *C. subradiosa* and *Loxoconcha* spp. are also recognized in the literature as among the most tolerant to environmentally stressful conditions [29,82]. Considering the length of time which passed between the samples being taken (1967, 2004-2017), and in the absence of an intermediate sampling activities to indicate with certainty the precise evolution of ostracod associations over in the last 50 years, it is at any rate possible to highlight how, since the nineteen-sixties, ostracods have been exposed to a potentially compromised environment due to the possible increase in anoxia phenomena [83], with few prevailing species tolerant to environmentally stressful conditions. Recent analysis of ostracod associations indicates a slight recovery in qualitative and quantitative terms in the early 2000s, with an increase in species adapted to high nutrient supply and tolerant to hypoxia. Later, from GTcRB to GTCrC, there is an evident decrease in ostracods, despite the presence of a marine protected area in the experimental site since 1986, with most of the identified species that show characteristics of opportunism and resistance to environmental stress

Furthermore, the rapid disappearance of Phanerogams, accelerated in 2015 and the strong decrease of the phytoplankton community in the late winter-early spring bloom observed in recent years (2010-2017) [77], from the GoT in general and in the experimental site in particular, could represent further evidence of the changed environmental conditions linked to phenomena of increasing anthropic stress and/or climatic change. These changes could partially explain the ostracod associations drop in GTCrC.

Finally, these results could confirm the possible environmental crisis linked to the recurrence of mucilage and hypoxic events, documented for the GoT between the 1980s and the first decade of the 21st century [74,75,76] as highlighted by the studies of molluscan and foraminiferal associations in the Bay of Panzano. The analyses of the ostracod associations in the same area confirm the above-mentioned environmental crises with a clear quali/quantitative decrease over the last 50 years (N. Pugliese personal communication, June 2020).

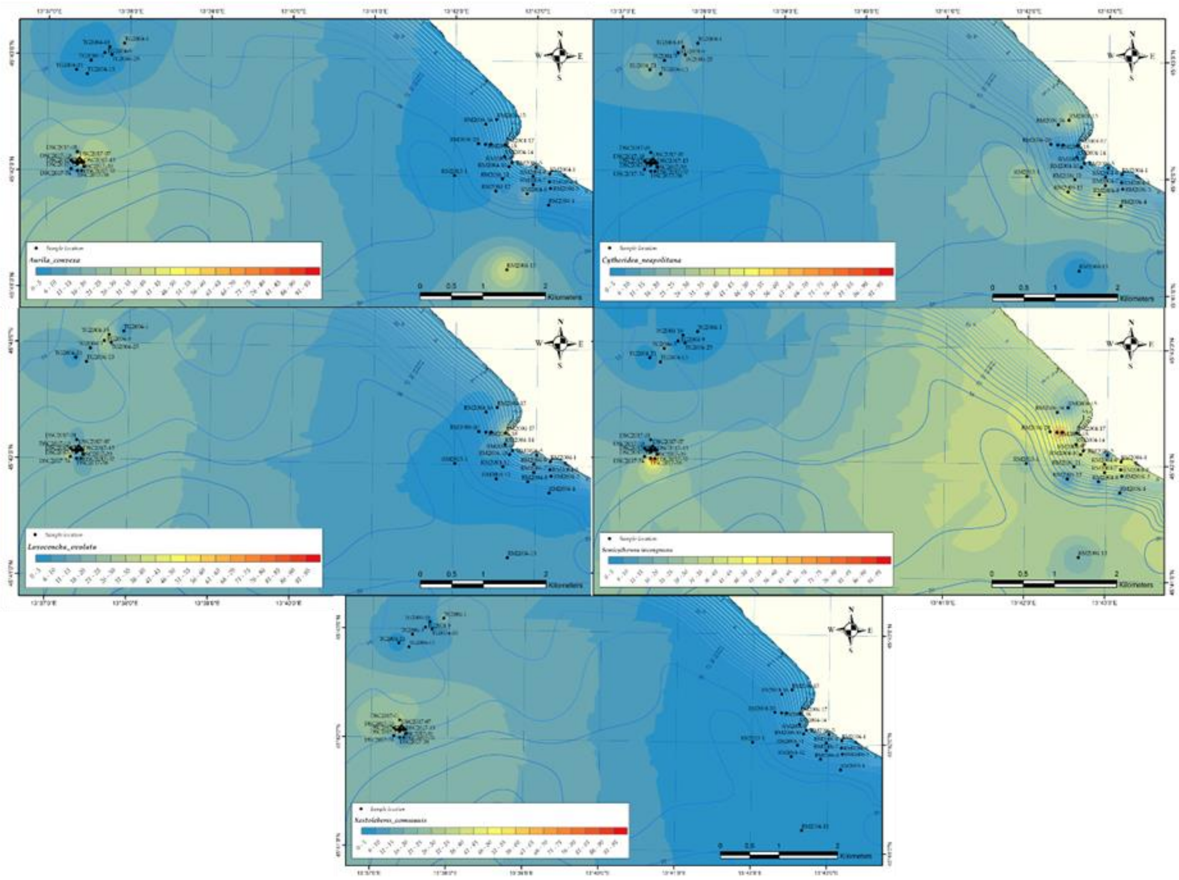

Figure 5. Diffusion of the opportunistic species in GTCrC interpolated in GIS using the Inverse Distance Weighting Method (IDW).

5. Conclusions

The analysis of ostracod associations found in the GoT during the period from 2004-2017 showed how over the last 20 years there has been a decline in environmental conditions with a clear

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decrease in quali/quantitative values. In particular, in GTCrB a higher number of species was observed than in GTCrC with a clear decrease in living specimens, but in both cases we highlighted a trend towards homogenization in the examined area with the domain of few species in most samples and in some cases samples were found to be monospecific. Most of the species recovered have characteristics of opportunism and tolerance to environmentally stressful conditions. *A. convexa, C. Neapolitana, Loxoconcha* spp., *S. incongruens* and *Xestoleberis* spp. are all known to be hypoxia-tolerant or opportunistic and able to survive in areas of severe environmental conditions such as the polluted Ex-Military Arsenal of La Maddalena Harbor [38].

The study on ostracod assemblages found in GTCrA, even when exercising caution with regards to a purely qualitative analysis, also record environmentally stressful conditions with the presence of few species often recognized in the literature as among the most tolerant to poor environmental conditions.

This data seems appears to be in agreement with the analysis performed in the GoT on other taxa (Mollusks and Foraminifera) thus confirming the possible environmental crisis linked to the recurrence of mucilage and hypoxic events, documented for the Gulf of Trieste between the 1980s and the first decade of the 21st century.

The ostracods decrease in GTCrC might be related to rapid disappearance of the Phanerogams, accelerated in 2015 and to the strong decrease of the phytoplankton community in recent years (2010-2017), markers of the changed environmental conditions linked to increasing anthropic stress and/or climatic change.

Finally, it must be underlined how the best environmental conditions in recent samples have been recorded in MPA. This is not entirely surprising since meiofauna are commonly early colonists, and that the most mobile and sensitive taxon rapidly colonize sediments after favorable conditions are restored [84]. Therefore, results from our study indicate that the preservation of large and connected patches of natural habitats is the most effective measure to halt further urbanization-driven biodiversity loss.

Future study on cores collected in selected areas of the GoT will improve understanding of the repercussions of anthropogenic activities over time on ostracod assemblages, as well as identifying additional indicator species, through seasonal sampling, that can be used to better define: a - possible causes of the recent decline in ostracod associations, b - the status and vulnerability of the ecosystem, c - to evaluate remediation activities to mitigate the negative impact of urbanization.

Author Contributions: Data Curation, conceptualization, methodology, writing-original draft and writing-review GG.S. and A.A.; conceptualization, methodology N.P. and S.C. (Saul Ciriaco); data curation, GIS processing M.F.; data curation marine survey M.C. (Massimo Celio); marine survey S.C. (Stefano Cirilli). All authors have read and agree to the published version of the manuscript.

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Appendix A

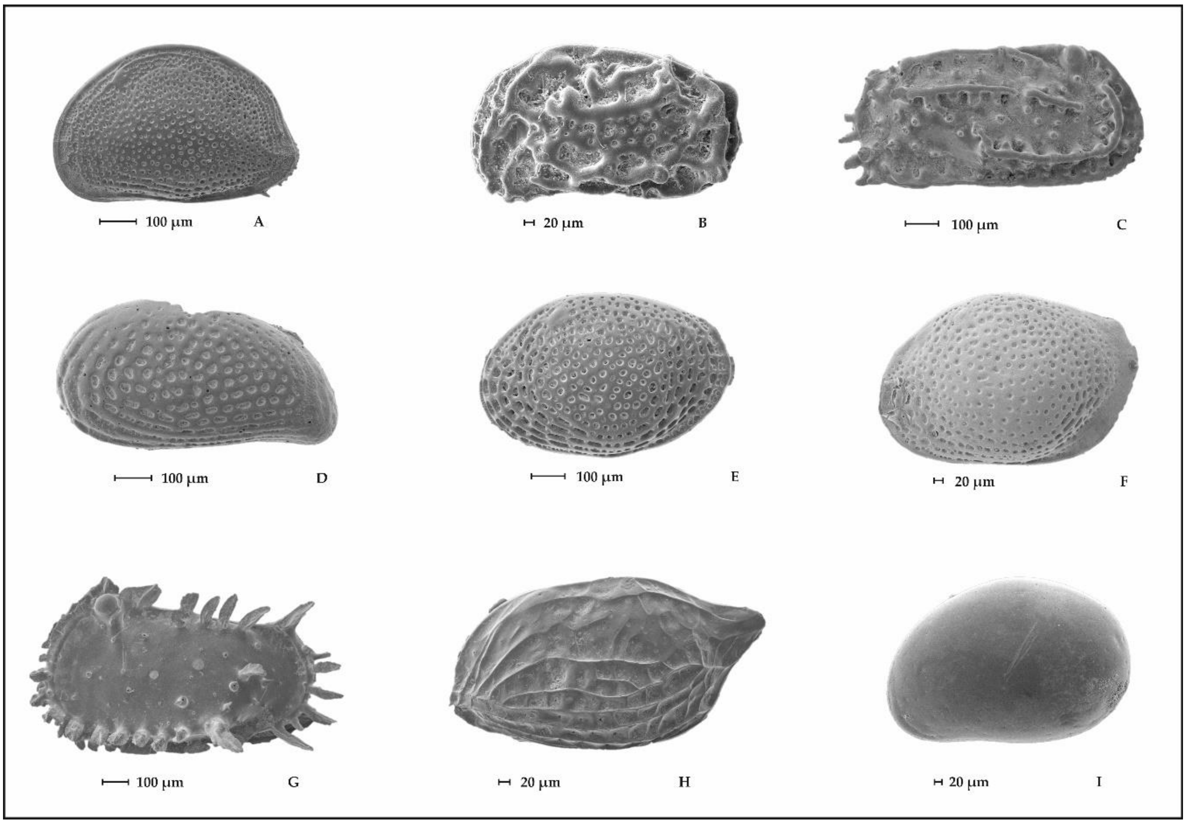

Plate A1: SEM photomicrographs of the GoT dominant ostracod taxa.

A. *Aurila convexa* (Baird, 1850). Left valve, lateral exterior view. Scale bar = 100µm;

B. *Callistocythere adriatica* Masoli, 1968. Left valve, lateral exterior view. Scale bar = 20µm;

C. *Carinocythereis whitei* (Baird, 1850). Right valve, lateral exterior view. Scale bar = 100µm;

D. *Cytheridea neapolitana Kollmann*, 1958. Right valve, lateral exterior view. Scale bar = 100µm;

E. *Loxoconcha ovulata* (Costa 1853). Left valve, lateral exterior view. Scale bar = 100µm;

F. *Loxoconcha rhomboidea* (Fischer, 1855). Left valve, lateral exterior view. Scale bar = 100µm;

G. *Pterygocythereis jonesi* (Baird, 1850) (Fischer, 1855). Left valve, lateral exterior view. Scale bar = 100µm;

H. *Semicytherura incongruens* (Müller, 1894) Ruggieri, 1959. Left valve, lateral exterior view. Scale bar = 20µm;

I. *Xestoleberis communis* (Müller 1894). Left valve, lateral exterior view, sample UC09. Scale bar = 20µm;

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