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Reconstructing Historical Changes in the Macroalgal Vegetation of a Central Mediterranean Coastal Area Based on Herbarium Collections

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Abstract: Well-conserved herbarium specimens of marine macroalgae represent a valuable resource for multiple types of investigation. When algal herbaria host specimens collected over long time spans from a certain geographic area, they have the potential to document historical changes in the benthic vegetation of that area. In this study, historical changes in the macroalgal vegetation of a central Mediterranean coast (Conero Riviera, Adriatic Sea) were assessed based on a critical re-examination of the herbarium of Irma Pierpaoli (collection period 1925–1951) and the phycological herbarium of the Polytechnic University of Marche (ANC ALG, collections made mostly in the period 1999–2024). For both herbaria, the identifications of many specimens were revised based on the current species circumscriptions. The comparison indicates that some major changes occurred between the two collection periods: a switch in the morphological functional structure of the vegetation (increase in the number of filamentous species, decrease in leathery macrophytes, and the near disappearance of calcareous articulated algae), local extinction of some species (at least 23, possibly more), and introduction of 11 species of non-indigenous seaweeds. Anthropogenic impacts (habitat destruction, increase in sediment load, and impacts of port activities and maritime traffic) are considered the main factors responsible for these changes.

Keywords: Adriatic Sea; algal herbaria; anthropogenic stressors; historical collections; long-term studies; macroalgal flora; marine macroalgae; non-indigenous species

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1. Introduction

Marine macroalgae have been collected and conserved as dried pressed specimens since the late 17th century. They attracted special interest in the Victorian period, when in Western Europe and North America, scientists as well as the middle-class public became fascinated by pressing seaweeds [1]. As a result, approximately one million and seven hundred thousand specimens are nowadays preserved in European herbaria (which, although considerable, is a significantly lower number when compared to vascular plants: [2,3].

In the past, herbarium specimens were used mostly for the traditional studies of algal taxonomy. Such use, in fact, has become even more frequent in the last twenty years due to the capacity to extract and sequence DNA from herbarium specimens and include such sequences in molecular phylogenetic datasets. There are many studies in which descriptions of new species [4], taxonomic revisions [5–7], biogeographic insights [8], and records of non-indigenous seaweeds [9,10] were based on DNA sequences obtained from herbarium specimens. In particular, in recent years, the development of high-throughput sequencing methods allowed sequences to be obtained even from the highly fragmented DNA of old herbarium specimens, such as type specimens. Sequences of type specimens allowed substantial advancements in the classification of several macroalgal genera (e.g., [11,12]), sometimes leading to the discovery of a great deal of cryptic diversity.

In recent years, the reconstruction of historical changes in the distribution of macroalgal species and assemblages has become a popular topic in investigations making use of algal herbaria (e.g., [2,13–23]). This interest is based on the fact that the distribution of many seaweed species is now shifting in response to climatic changes at regional and global scales [24–27]. Predicting future changes in the distribution of seaweeds is essential to plan measures for the management of benthic coastal communities [1]. Such information is especially important for large canopy-forming macroalgae and seagrasses due to their habitat-forming nature and the many ecological functions played by these organisms in coastal benthic habitats [27,28]. Such planning, however, requires historical data to establish more informed ecological baselines. In this regard, well-curated herbarium collections have the potential not only to document changes in the past geographic distribution, but also correlate changes in features of herbarium specimens with environmental factors that might have influenced these changes. For example, examining the herbarium specimens of *Gelidium* collected over the period 1878–2018, Miller et al. [1] demonstrated a correlation between the content of nitrogen isotopes ($\delta^{15}N$) in the tissues of these algae and the productivity regime of southern Monterey Bay from 1946 to 2018.

Rocky shores located in or near urban areas are among the most affected by anthropogenic impacts, and their benthic vegetation has often substantially changed in response to such impacts. The Conero Riviera can be considered an example of this situation. This area, located in the central Adriatic shore of Italy, is a 20 km long rocky coast affected by the urban influences of the port city of Ancona. The macroalgal flora of the Conero Riviera has been generally understudied in comparison to the rest of the Mediterranean. Detailed information, however, is available for the years 1925–1950 thanks to the work of Irma Pierpaoli [29,30]. Her collections produced a herbarium consisting of 661 specimens from the Adriatic and Ionian seas [22], most of which were collected from the Ancona area. These specimens provide good documentation of the macroalgal flora at that time [31,32]. The subsequent studies of macroalgae in this area are more recent and have been carried out in the periods 1964–1976, 1997–1999 (collections by Attilio Solazzi and collaborators), and, mainly, in the period 2012-present (collections by Fabio Rindi and collaborators) [33]. Most of the specimens collected in these surveys are now deposited in the herbarium ANC ALG, the phycological section of the Herbarium Anconitanum (ANC), Polytechnic University of Marche.

In 2020, Rindi et al. [33] assessed long-term changes in the macroalgal flora of the Conero Riviera based on the information available at that time. Since that study, however, many new surveys have been conducted and many new collections have been included in ANC ALG. In this study, we critically revise the identifications of the specimens in both the Pierpaoli and ANC ALG herbaria (rejecting, for several, the identifications provided in previous studies). We present here a focused reassessment of the changes in the macroalgal vegetation of this area based on information derived from the re-examination of these herbaria.

2. Materials and Methods

2.1. Study Area

The geographic area considered in this study is a stretch of shore about 25 km long spanning from the Porticciolo di Torrette to the town of Numana, on the Adriatic coast of Italy (Figure 1). This area includes the city of Ancona, the suburb of Torrette, and the Conero Riviera (the stretch of rocky shore located South of Ancona). For this study, we considered specimens of the Pierpaoli and ANC ALG herbaria collected from this area. Details of the geomorphological and oceanographic features of the area are available in Rindi et al. [33].

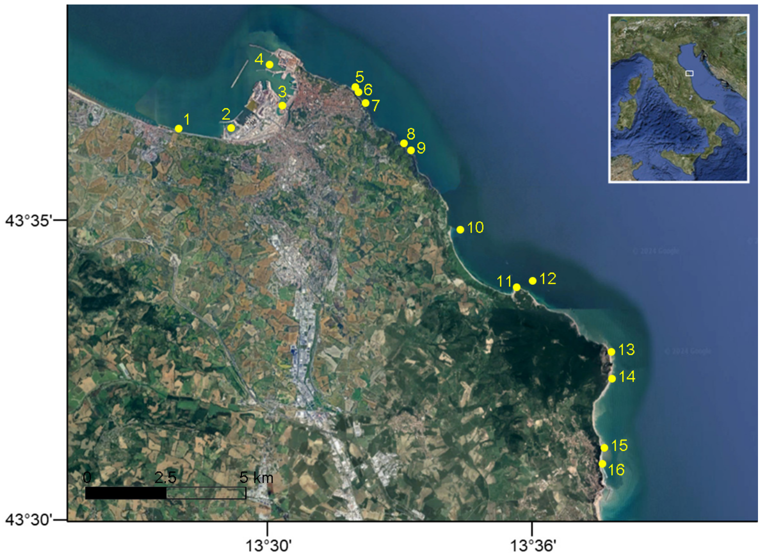

Figure 1. Map of the study area. The collection sites for the specimens deposited in ANC ALG are indicated by numbers referring to the sites (see list in Table S2 for details of the sites).

For the long-term comparison presented in this study, the shore called Passetto di Ancona is of special interest, because numerous specimens deposited in the Pierpaoli and the ANC ALG herbaria were collected from this site (Tables S1–S4). This is a 1.5 km long urban rocky shore located on the Eastern side of the promontory of Ancona, at the bottom of steep cliffs. Here, stretches of wave-exposed shore alternate with stretches partially sheltered by shallow reefs; in a part of the area, some rockpools of variable extent and depth are present. Whereas in the years of Pierpaoli's collections the coast consisted entirely of natural rock (marl), today a large part is artificial, being formed by a concrete pavement and large boulders deployed for coastal protection (Figure 2).

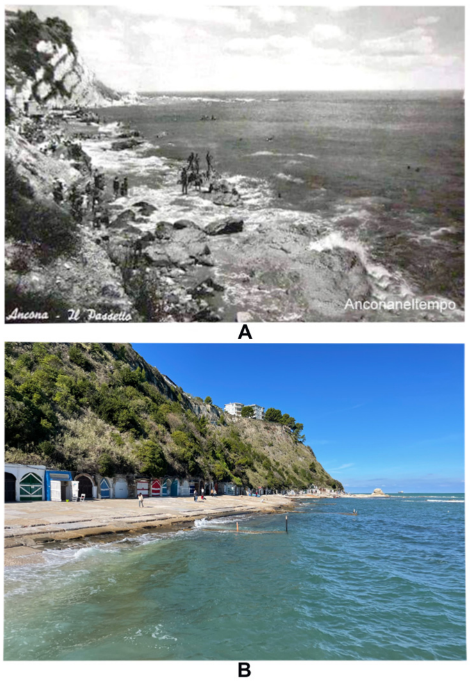

Figure 2. (**A**) The shore of Passetto di Ancona in the 1930s. Postcard obtained from the Facebook page Ancona nel tempo (https://www.facebook.com/groups/anconaneltempo; accessed on 10 October 2024). (**B**) The same shore at the present time (October 2024).

2.2. The Pierpaoli Herbarium

Irma Pierpaoli was a school teacher who lived in Marche (the region of Ancona) from 1925 until her passing in 1967 [32]. During this period, she examined with great dedication samples of marine macroalgae, collected either by herself or other collectors (mainly fishermen who donated to her material attached to fishing gear). These specimens are now conserved in the Pierpaoli Herbarium, which is stored at the headquarters of the Italian National Research Council in Taranto. The Pierpaoli herbarium includes 393 specimens collected in the area covered by this study in the period 1925–1951 (mostly in the years 1930–1942). The Pierpaoli herbarium was the object of a taxonomic and nomenclatural revision by Cecere and Saracino [31] and Cecere et al. [32]. For many specimens in the herbarium, the collection site and date were marked on the sheets by Pierpaoli. For the remaining, however, some details are missing (either the site or the collection date, or both, are not reported). If the site is missing but the specimen is mounted on the same sheets with specimens explicitly indicated from the area of Ancona, it has been assumed that their provenance was from the same area. In other cases, the collection details are reported only in a general form (for example, the collection site is reported as port, without specification of the exact place within the port; or only the year is reported without the exact date). Table S1 shows a list of the sites or location indications provided by Pierpaoli for her specimens.

2.3. The ANC ALG Herbarium

The ANC ALG herbarium is a separate section of the ANC herbarium and includes specimens of macroalgae collected by the staff of the Polytechnic University of Marche. It is stored in the Department of Life and Environmental Sciences of this university. The collections of the period 1997–1999 were made by Attilio Solazzi, former Professor of Botany at the university, and his students. The collections from 2012 to the present were made by Fabio Rindi and collaborators in the context of surveys carried out for several research projects. The ANC ALG herbarium currently hosts about 1200 specimens collected from several regions of Italy (mainly Marche, Veneto, Apulia, and Sardinia), plus some additional collections from other European countries. At present, 622 specimens have been cataloged and numbered; these include nearly all the specimens collected from the area considered in this study. For them, information on the collection sites (including GPS coordinates, when available) and collection date is available and reported on the herbarium labels. The details for the ANC ALG collection sites are included in Table S2.

2.4. Procedure Used for the Long-Term Comparison

All the sheets of the Pierpaoli herbarium with specimens from the Conero Riviera were inspected and photographed. All the collection data were annotated. The specimens of the ANC ALG herbarium were directly re-examined. Based on this revision, for both herbaria, the identifications were either confirmed or revised. For the Pierpaoli herbarium, Cecere and Saracino [31] and Cecere et al. [32] reassessed the species identifications based on the accepted taxonomy at the time of these studies. In many cases, we accepted the identifications provided by these authors, but for some specimens, we propose here new identifications (Table S3). The same was carried out for the ANC ALG herbarium (Table S4); in this case, revised identifications concerned mostly specimens of some genera of red and green algae (*Asparagopsis*, *Ceramium*, *Pachymeniopsis*, *Vertebrata*, and *Ulva*) for which DNA sequence data were generated (and will be presented in a separate manuscript; Giulia Bellanti and Fabio Rindi, in preparation). A comparative list of the taxa/species for which specimens are deposited in the two herbaria is presented in Table S5.

Based on the data derived from the two herbaria and additional information obtained from the relevant literature [29,30,33–39], a critical comparison of the flora of the Pierpaoli period and the contemporary flora is presented in Table 1. The algal species documented in the two herbaria were subdivided into morphological functional groups following Steneck and Dethier [40] (adding a group defined as tubular for the species of *Ulva* formerly classified as *Enteromorpha*, which was not referable to any of the groups proposed by these authors). Taxonomy and nomenclature in this paper follow [41].

Table 1. Floristic lists for the study area based on information derived from the Pierpaoli and ANC ALG herbaria and the literature. + indicates species for which specimens are deposited in the corresponding herbaria; (+) denotes species that have been mentioned in the literature, but for which no specimens have been deposited. † denotes species present in the Pierpaoli period that have now disappeared. Species that have been introduced into the study area after the Pierpaoli period are underlined. Abbreviations for the morphological functional (M/F) groups of algae: AC = articulated calcareous; Cru = crustose; Fil = filamentous; CF = corticated foliose; CM = corticated macrophyte (terete); LM = leathery macrophyte; Tub = tubular.

Species (Current Accepted Identification)	M/F Group	Pierpaoli Period Flora	Contemporary Flora
Magnoliophyta			
Cymodocea nodosa			+
Zostera marina		(+)	+
Chlorophyta			
Acetabularia acetabulum	Fil	+	+
Bryopsis corymbosa	Fil	(+)	+
Bryopsis cupressina	Fil	+	
Bryopsis cupressina var. *adriatica*	Fil		+
Bryopsis duplex	Fil		+
Bryopsis feldmannii	Fil	+	(+)
Bryopsis hypnoides	Fil	+	+
Bryopsis muscosa	Fil	+	
Bryopsis pennata	Fil		+
Bryopsis plumosa	Fil	+	+
Chaetomorpha aerea	Fil	+	+
Chaetomorpha ligustica	Fil	+	(+)
Chaetomorpha pachynema	Fil		+
Cladophora coelothrix	Fil		+
Cladophora dalmatica	Fil		(+)
Cladophora flexuosa	Fil	+	
Cladophora hutchinsiae	Fil		+
Cladophora laetevirens	Fil		+
Cladophora lehmanniana	Fil		+
Cladophora prolifera	Fil	+	+
Cladophora vagabunda	Fil		+
Codium fragile	CM		+
Codium vermilara †	CM	+	
Dasycladus vermicularis †	CM	+	
Derbesia tenuissima	Fil		+
Flabellia petiolata †	CF	(+)	
Halimeda tuna †	AC	+	
Ulva compressa	Tub	(+)	+
Ulva flexuosa	Tub		(+)
Ulva intestinalis	Tub	+	(+)
Ulva lacinulata	Fol	+	+
Ulva linza	Fol	+	+
Ulva prolifera	Tub	+	
Ulva rigida	Fol		+
Ulvella lens	Cru		(+)
Ulvella viridis	Cru		(+)
Rhodophyta			
Aglaothamnion feldmanniae	Fil		(+)
Aglaothamnion scopulorum	Fil	(+)	
Alsidium corallinum	CM	+	+

Table 1. *Cont.*

Species (Current Accepted Identification)	M/F Group	Pierpaoli Period Flora	Contemporary Flora
Amphiroa rubra †	AC	+	
Antithamnion cruciatum	Fil	+	+
Antithamnion hubbsii	Fil		+
Antithamnionella elegans	Fil		+
Antithamnionella spirographidis	Fil		+
Asparagopsis taxiformis	Fil		+
Balliella cladoderma	Fil	(+)	
Bangia fuscopurpurea	Fil	+	+
Botryocladia botryoides †	CM	(+)	
Callithamnion corymbosum	Fil		+
Callithamnion granulatum †	Fil	(+)	
Carradoriella denudata	Fil		+
Centroceras gasparrinii	Fil		(+)
Ceramium cimbricum	Fil	+	
Ceramium circinatum	Fil	+	(+)
Ceramium derbesii	Fil	+	+
Ceramium diaphanum	Fil	+	+
Ceramium nudiusculum	Fil	+	+
Ceramium secundatum	Fil		+
Ceramium siliquosum	Fil		+
Ceramium cf. *polyceras*	Fil		+
Champia parvula	CM	+	(+)
Chondracanthus acicularis	CM	+	+
Chondria capillaris	CM	(+)	+
Chondria mediterranea	CM		+
Chondria dasyphylla	CM	+	+
Chondrymenia lobata †	CF	+	
Chrysymenia ventricosa †	CM	(+)	
Colaconema daviesii	Fil		+
Colaconema nemalii	Fil	(+)	(+)
Compsothamnion thuyoides	Fil		+
Contarinia peyssonneliiformis	Cru	(+)	
Corallina berteroi	AC	+	+
Cryptonemia palmetta	CF	(+)	+
Cryptopleura ramosa	Fol	(+)	
Dasya pedicellata	Fil		+
Erythrotrichia carnea	Fil	+	(+)
Erythrotrichia investiens	Fil	(+)	(+)
Gastroclonium clavatum	CM	+	+
Gastroclonium reflexum	CM		+
Gayliella mazoyerae	Fil		(+)
Gelidium corneum	CM	(+)	
Gelidium crinale	CM	+	+
Gelidium minusculum	CM		+
Gelidium pusillum	CM	+	+
Gelidium spathulatum	CM	+	+
Gelidium spinosum	CM		+
Gracilaria armata	CM	+	+
Gracilaria bursa-pastoris	CM	+	+
Gracilaria dura	CM	+	+
Gracilaria gracilis	CM		+
Gracilaria longa	CM	+	
Gracilariopsis longissima	CM	+	
Grateloupia filicina	CM	+	+
Griffithsia schousboei	Fil	(+)	
Gymnogongrus griffithsiae	CM		+
Halopithys incurva	CM	+	+
Halymenia floresii	CF	(+)	+
Haraldia lenormandii	Fol	(+)	

Table 1. *Cont.*

Species (Current Accepted Identification)	M/F Group	Pierpaoli Period Flora	Contemporary Flora
Herposiphonia tenella	Fil		+
Hydrolithon farinosum	Cru	+	(+)
Hypnea cervicornis	CM		+
Hypnea musciformis	CM	+	+
Jania longifurca †	AC	(+)	
Jania rubens †	AC	(+)	
Jania virgata †	AC	(+)	
Laurencia obtusa	CM	+	+
Liagora viscida	CM		+
Lithophyllum cystoseirae	Cru	(+)	
Lithophyllum incrustans	Cru	(+)	
Lomentaria articulata	CM	+	
Lomentaria clavellosa	CM	+	+
Lomentaria compressa	CM	+	+
Lomentaria firma	CM	+	
Lophosiphonia obscura	Fil		+
Melanothamnus japonicus	Fil		+
Melobesia membranacea	Cru		(+)
Monosporus pedicellatus	Fil	(+)	
Nemalion lubricum	CM	+	+
Nemastoma dichotomum †	CM	+	
Nitophyllum punctatum	Fol	+	+
Osmundaria volubilis †	CF	(+)	
Osmundea pinnatifida	CM		(+)
Osmundea truncata	CM	+	+
Osmundea verlaquei	CM		+
Pachymeniopsis cf. *lanceolata*	CF		+
Palisada patentiramea	CM		+
Palisada perforata	CM	+	+
Palisada thuyoides	CM	(+)	+
Peyssonnelia bornetii	Cru	+	+
Peyssonnelia dubyi	Cru		(+)
Peyssonnelia rubra	Cru	+	+
Peyssonnelia squamaria	Cru		+
Pleonosporium borreri	Fil		+
Polysiphonia devoniensis	Fil		(+)
Polysiphonia divergens	Fil	(+)	
Polysiphonia morrowii	Fil		+
Polysiphonia opaca	Fil	(+)	+
Polysiphonia sanguinea	Fil		+
Polysiphonia sertularioides	Fil	(+)	+
Polysiphonia vinosa	Fil		+
Porphyra cf. *umbilicalis*	Fol	+	
Pseudoceramium tenerrimum	Fil		+
Pterocladiella capillacea	CM		+
Pterocladiella melanoidea	CM		+
Pterothamnion crispum	Fil	(+)	
Pterothamnion plumula	Fil	(+)	(+)
Pyropia elongata	Fol	+	+
Radicilingua reptans	Fol		+
Radicilinga thysanorizans	Fol		+
Rhodophyllis divaricata	Fol	+	+
Rhodymenia ardissonei	CF	+	+
Rytiphlaea tinctoria	CM	+	+
Schottera nicaeensis	CF		+
Scinaia furcellata	CM		+
Seirospora sphaerospora	Fil		+
Spermothamnion repens	Fil		+
Spyridia filamentosa	Fil	(+)	+

Table 1. *Cont.*

Species (Current Accepted Identification)	M/F Group	Pierpaoli Period Flora	Contemporary Flora
Stylonema alsidii	Fil		(+)
Thuretella schousboei	CM	+	+
Titanoderma pustulatum	Cru	(+)	(+)
Tricleocarpa fragilis †	AC	(+)	
Vertebrata fruticulosa	Fil	+	+
Vertebrata furcellata	Fil		+
Vertebrata martensiana	Fil		+
Wrangelia penicillata †	Fil	+	
Yendoa hakodatensis	CM		+
Yoneshiguea compta	Fil		+
Heterokontophyta–Phaeophyceae			
Arthrocladia villosa †	CM	(+)	
Asperococcus ensiformis	CM	+	+
Asperococcus fistulosus	CM		+
Cladostephus hirsutus	Fil	+	+
Colpomenia peregrina	CM		+
Cutleria multifida	CF	+	+
Cystoseira compressa	LM	+	+
Cystoseira humilis †	LM	+	
Cystoseira humilis var. *miriophylloides*	LM		+
Dictyopteris polypodioides	CF	+	+
Dictyota cyanoloma	CF		(+)
Dictyota dichotoma	CF	+	+
Dictyota dichotoma var. *intricata*	CF	+	+
Dictyota fasciola	CF	+	
Dictyota implexa	CF	(+)	+
Ectocarpus siliculosus	Fil	(+)	+
Ericaria dubia †	LM	(+)	
Feldmannia mitchelliae	Fil	(+)	+
Feldmannia cf. *lebelii*	Fil		+
Feldmannia cf. *padinae*	Fil		+
Fucus virsoides †	LM	+	
Gongolaria barbata	LM	+	+
Gongolaria barbata f. *hoppei*	LM	+	
Halopteris scoparia	Fil	+	+
Herponema velutinum	Fil		(+)
Hincksia dalmatica	Fil		+
Hincksia mitchelliae	Fil		(+)
Hincksia secunda	Fil		+
Myrionema strangulans	Cru		+
Nereia filiformis	CM		+
Padina pavonica	CF	+	+
Petalonia fascia	CF	(+)	+
Pseudolithoderma adriaticum	Cru		(+)
Sargassum acinarium †	LM	+	
Sargassum hornschuchii †	LM	+	
Sargassum muticum	LM		+
Scytosiphon dotyi	CM		+
Scytosiphon lomentaria	CM	+	+
Sphacelaria cirrosa	Fil		+
Sphacelaria fusca	Fil		+
Sphacelaria rigidula	Fil		(+)
Sphacelaria tribuloides	Fil		+
Stictyosiphon adriaticus	Fil		+
Striaria attenuata †	CM	+	
Taonia atomaria	CF		+
Taonia pseudociliata	CF		+

3. Results

3.1. Numbers and Identifications of Specimens in the Pierpaoli and ANC ALG Herbaria

The numbers of specimens, identifications, and collection details for the Pierpaoli and ANC ALG herbaria are shown in Tables S3 and S4, respectively. Table S5 presents a comparative list of the taxa documented in the two herbaria, including collection details.

Based on our revision, the Pierpaoli herbarium includes 393 specimens collected from the study area (Table S5). These belong to 87 macroalgae identified at least at the genus level (16 green algae, 53 red algae, and 18 brown algae); for 82 of them (16 green algae, 48 red algae, and 18 brown algae) the identification is at the species or subspecific level. The specimens were collected mostly from two areas: the port of Ancona and the area of Passetto di Ancona (Table S1). For most of the Pierpaoli specimens, we accept the identifications proposed by Cecere and Saracino [31] and Cecere et al. [32], as marked on the herbarium labels. For 69 specimens, however, we propose revised identifications (Table S3). It is noteworthy that in her two publications, Pierpaoli [29,30] cited 51 species (listed in Table S6) for which we did not find any specimens in the herbarium.

The ANC ALG herbarium currently includes 590 specimens collected in the study area in the years 1966–2024 (the vast majority in the years 2012–2024, Table S4). Of these, 524 have been identified at the species or subspecific level and belong to 134 species (2 angiosperms, 21 green algae, 81 red algae, and 30 brown algae). Revised identifications are proposed here for 27 specimens (Table S4). After a critical re-examination, we conclude that 53 species (7 green algae, 34 red algae, and 12 brown algae) are certainly common to the two herbaria and were present in the study area in both collection periods.

3.2. Temporal Variation in the Macroalgal Vegetation of the Area of Ancona

Based on a critical reconsideration of the herbarium and literature information, we conclude that the flora of the Pierpaoli period consists of 124 species/taxa (20 green, 80 red, and 24 brown), whereas the contemporary flora consists of 166 species/taxa (28 green, 101 red, and 37 brown) (Table 1).

In terms of morphological functional groups, filamentous algae (39 species) and corticated macrophytes (terete) (38 species) were the two most represented groups in the Pierpaoli flora, followed by corticated flattened algae (14 species). This pattern is common to the contemporary flora, where, however, filamentous algae (78 species) were remarkably dominant over all the other groups. Even in this case, corticated macrophytes (terete) (46 species) and corticated flattened algae (15 species) were the other two most abundant groups. The relative abundances of the morphological functional groups in the two floras are represented in terms of percentage in Figure 3.

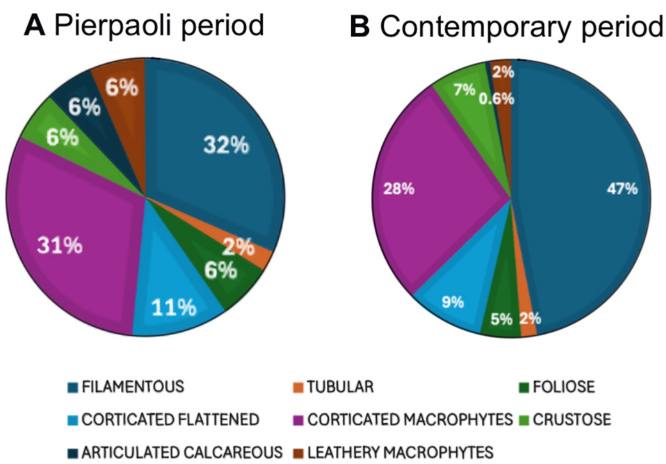

Figure 3. Percentage representation of morphological functional groups for the Pierpaoli period and the contemporary period. (**A**) Pierpaoli period. (**B**) Contemporary period.

4. Discussion

4.1. Comparison of Pierpaoli and ANC ALG Herbaria

Our critical re-examination showed a large number of species in common between the Pierpaoli and the ANC ALG herbaria, but also a difference in the number of species represented (79 in Pierpaoli and 134 in ANC ALG). The same point applies to the comparison between the Pierpaoli and the contemporary floras based on the herbarium and literature information (124 for the Pierpaoli period and 166 for the contemporary period). Notably, the 166 species that we document here for the contemporary flora is a higher number than the 133 reported by Rindi et al. [33] for the years 2010–2019 (for an area that was not exactly the same as the one covered by this study, but largely overlapped with it). The 33 additional species are the result of surveys carried out after 2020 (in which many new specimens were deposited in ANC ALG). There are three main reasons for the discrepancy between the Pierpaoli flora and the contemporary flora. The first is uncertainty related to the identification of some specimens and difficulty in establishing a correspondence between the Pierpaoli specimens and ANC ALG specimens. This problem concerns some algal genera for which identification is notoriously difficult (e.g., *Bryopsis*, *Cladophora*, *Ceramium*, *Gelidium*, *Pyropia*, and *Ulva*). In these algae, the circumscription of species boundaries based only on morphological characters is problematic; an accurate identification requires DNA barcoding data (e.g., [42–45]). For the Pierpaoli herbarium, identifications have to be based necessarily on morphological features, so for some specimens, a margin of uncertainty remains. We decided to reassess the identity of some of Pierpaoli's specimens based on information gained from DNA sequence data obtained from contemporary samples. These include, for example, specimens that we refer to as *Ulva lacinulata* [46], *Ceramium derbesii*, *Ceramium nudiusculum*, and *Pyropia elongata*. It should also be considered that classification, taxonomy, and species concepts at the time of Pierpaoli's work were substantially different from the current ones; in addition, the quality of the microscopes available to Pierpaoli was certainly not comparable with the present and did not allow similarly detailed observations. In consideration of these problems, it cannot be excluded that the number of species common to the two herbaria might be higher.

Secondly, the coverage in terms of spatial sampling is not the same for the two herbaria and sampling periods. Pierpaoli collected many samples from the port of Ancona, at sites that today are no longer accessible, have been deeply modified, or have been destroyed (and therefore, have not been resampled in recent years). An interesting example is the site called San Clemente, or Scogli di San Clemente (Table S1). This was a group of four rocks that until World War II emerged near the entrance of the port of Ancona. They were subsequently covered and paved under the Fincantieri shipyard, and today no longer exist. Remarkably, Pierpaoli collected many specimens from this site, including some species that have never been found again (e.g., the green alga *Halimeda tuna*; Figure 4). Conversely, numerous specimens deposited in ANC ALG have been collected in the last 25 years from sites of the Conero Riviera that can be accessed easily only by boat from the sea (Spiaggia della Scalaccia, Secca dell'Ospedale, Spiaggia dei Sassi Neri, and Spiaggia delle Due Sorelle) and were not sampled by Pierpaoli. Furthermore, Pierpaoli did not have the snorkeling or SCUBA diving equipment available today, so she could not collect several subtidal species that are represented in ANC ALG (especially small filamentous seaweeds). The only site for which the sampling effort is somewhat comparable is Passetto di Ancona. Collections from this area include specimens of 55 species in the Pierpaoli herbarium and 88 species in the ANC ALG herbarium (Table S7). Such difference does not indicate an increase in seaweed diversity and is certainly due to increased and more targeted sampling efforts in contemporary surveys (favored by easier access to subtidal habitats for modern investigators). Despite this, the herbarium records indicate that several species with distinctive morphologies (e.g., *Cladostephus hirsutus*, *Chondrymenia lobata*, *Dasycladus vermicularis*, *Lomentaria* spp., *Nemastoma dichotomum*, *Peyssonnelia bornetii*, and *Peyssonnelia rubra*) disappeared from the shore of the Passetto. Thirty species are represented in both herbaria (Table S7); notably, still today, these are the most common and abundant species in this area (Fabio Rindi and Giulia Bellanti, personal observation).

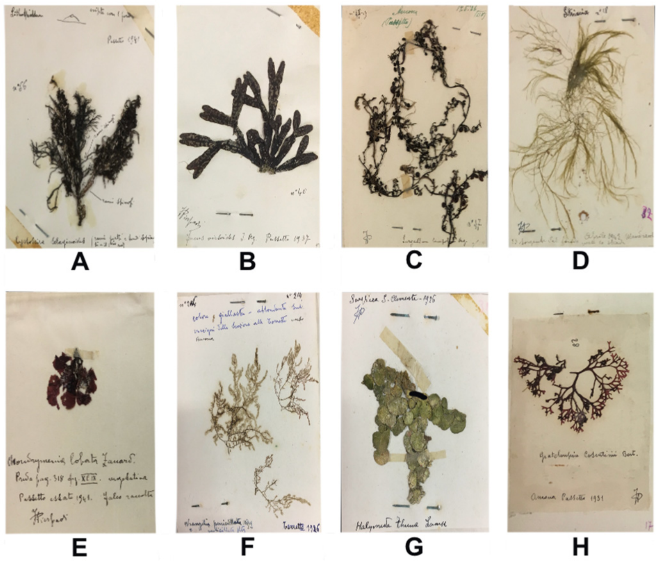

Figure 4. Specimens of the Pierpaoli herbarium documenting species that have disappeared in the contemporary flora. (**A**) *Cystoseira humilis*. (**B**) *Fucus virsoides*. (**C**) *Sargassum acinarium*. (**D**) *Striaria attenuata*. (**E**) *Chondrymenia lobata*. (**F**) *Wrangelia penicillata*. (**G**) *Halimeda tuna*. (**H**) *Nemastoma dichotomum*.

Finally, other differences between the two herbaria are due to some real changes in the flora that took place in the period between the two collection phases. This aspect is discussed below.

4.2. Long-Term Changes in the Macroalgal Vegetation of the Study Area and Factors That Caused Them

The critical revision of the two herbaria confirms that the local extinction of some species and the introduction of some non-indigenous seaweeds took place between the Pierpaoli period and the contemporary period, as suggested by Rindi et al. [33]. At least 23 seaweed species disappeared between the two periods, resulting in their absence in the ANC ALG herbarium (Table 1). The morphology of these algae is distinctive and makes them easy to recognize in the field, so we consider it impossible that they may have been missed; despite intensive surveys, they have not been rediscovered in recent decades (Figure 4).

Other species documented or mentioned by Pierpaoli (approximately 15) have also not been found in recent decades (Table 1). Most of these, however, are small seaweeds with filamentous or foliose morphologies, so we do not deny that they may have been missed and will be recollected in the future. In terms of the morphological functional structure, there has been a major increase in the number of filamentous species (Figure 3). Filamentous and foliose algae are opportunistic, fast-growing organisms with large reproductive outputs, which are generally more tolerant to anthropogenic stressors than large perennial algae [40,47–49]. In conjunction, the species of leathery macrophytes have halved (from 8 to 4) and articulated calcareous forms have nearly disappeared (from 7 to 1, although the only species left, *Corallina berteroi*, is still widely distributed along the Conero Riviera). Leathery macrophytes are perennial seaweeds and include species highly sensitive to anthropogenic disturbances [50–52]. The rarefaction of these algae (especially species of the *Cystoseira s.l.* complex) has been reported throughout the Mediterranean [53–57], although it is not always clear which are the key stressors that determined it (e.g., [58]).

We argue that urbanization, mainly through habitat destruction and increase in sediment load and chemical pollution, is the main disturbance that determined the species loss between the two sampling periods. During and after World War II, the area of the port of Ancona was deeply modified and the port infrastructure was greatly expanded (in particular, large portions of the seabed on the western side of the promontory of Ancona were paved and are now occupied by wharfs, docks, and other port facilities; see Figure S2 for a comparison). This certainly produced an increase in sediment load, water turbidity, and the amount of pollutants generated by the increased maritime traffic. These are major impacts for marine macroalgae in general, and particularly for habitat-forming brown algae (as reflected by the loss of five leathery macrophytes, i.e., *Cystoseira humilis*, *Ericaria dubia*, *Fucus virsoides*, *Sargassum acinarium*, and *Sargassum hornschuchii*). In addition, habitat modification at some sites outside the port (e.g., the Passetto) greatly facilitated access to the shoreline, resulting in a strong impact of trampling in the summer months. Frequent trampling has a strong mechanical impact on coastal communities and may result in the local losses of algae with fleshy thalli [59,60]. It cannot be excluded, however, that some changes at the basin scale in the physico-chemical and oceanographic features of the study area may have also contributed to the losses documented by the two herbaria. Changes of this type have been invoked as a potential cause of the regression of *Fucus virsoides* in the northern Adriatic [58], a species that disappeared from Ancona probably in the 1950s or 1960s. The coastal area of Ancona is located in the southern part of the northern Adriatic sub-basin. The northern Adriatic Sea is a mid-latitude shallow continental shelf strongly impacted by river discharge (mainly the Po River), human activity, and climate change that certainly determined long-term changes in the macroalgal vegetation [61]. However, long-term data series of physical and biogeochemical parameters were analyzed only from the 1970s in this area [62], i.e., after Pierpaoli's work. Over the past four decades, an increase in sea surface temperature has been observed, as well as a marked change in the annual mean flow of the Po River due to periods of persistent drought. Moreover, a long-term decrease in surface phosphate concentrations in both Po River water and seawater was observed, linked to the removal of polyphosphates in detergents by legislative acts in the period 1985–1990 [63] and the lower use of phosphate-based fertilizers in agriculture [64,65]. However, a significant increase in nitrate concentration in seawater was observed. In this area, given that some relationship between these environmental changes and the changes in the community structure and seasonal trends of phytoplankton has been observed [66,67], it is reasonable to think that these changes have also affected, to some extent, the macroalgae community.

The introduction of some non-indigenous seaweeds is the other obvious change in the macroalgal vegetation that took place in the years between the Pierpaoli period and the contemporary period (Figure 5). The non-indigenous nature of these species in the Mediterranean is well documented, and nine of them (*Aglaothamnion feldmanniae*, *Antithamnion hubbsii*, *Codium fragile*, *Hypnea cervicornis*, *Melanothamnus japonicus*, *Pachymeniopsis* cf. *lanceolata*, *Polysiphonia morrowii*, *Sargassum muticum*, and *Yendoa hakodatensis*) were already recorded for the area of Ancona [33,35,36]. Two additional ones are newly reported here and documented in ANC ALG: *Colpomenia peregrina* (two specimens, collected in 2021 and 2024) and *Asparagopsis taxiformis* (three specimens of the sporophyte, the *Falkenbergia* phase, collected in 2023 and 2024). These are not unexpected discoveries, since the presence of these algae in the Mediterranean has been documented in other localities [68–71]. *Asparagopsis taxiformis* is probably a recent arrival in the study area, where it was first noted in early 2023 (Fabio Rindi, personal observation). *Colpomenia peregrina* has likely been present for a longer time, but it may have gone unnoticed due to the habitat and site where it was discovered (Molo Nord in the port of Ancona, in the canopy of the other introduced brown alga *Sargassum muticum*).

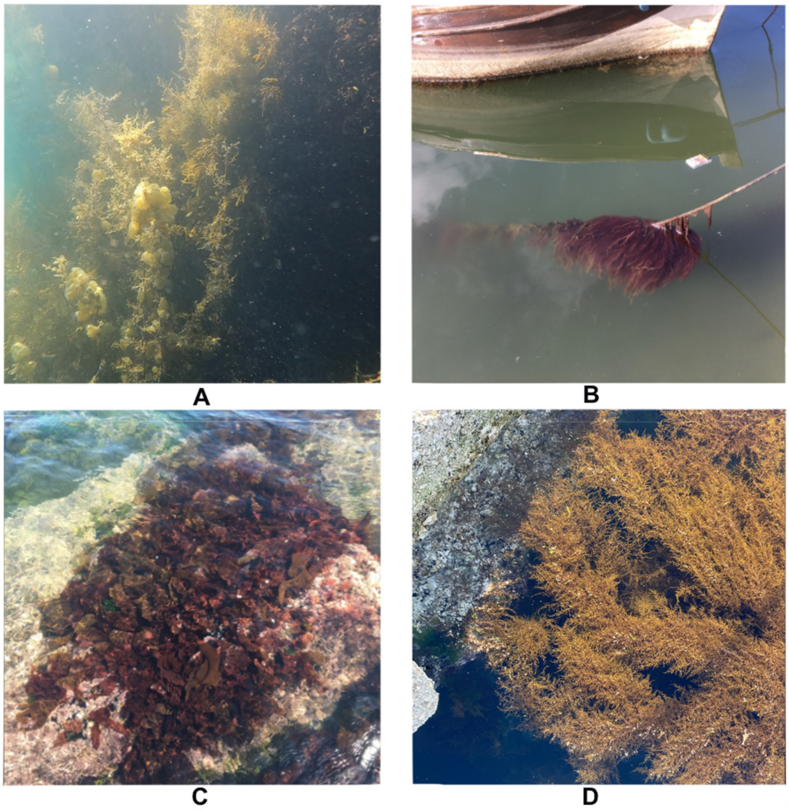

Figure 5. Field specimens of some species that were introduced in the study area in the period between the Pierpaoli collections and the contemporary collections. (**A**) *Colpomenia peregrina*, epiphytic on *Sargassum muticum*. (**B**) *Polysiphonia morrowii*. (**C**) *Pachymeniopsis* cf. *lanceolata*. (**D**) *Sargassum muticum*.

5. Conclusions

Even considering the differences in spatial coverage and the uncertainties about the identification of some of Pierpaoli's specimens, we believe that the two herbaria provide a good representation of the macroalgal flora of the study area in the two collection periods. In particular, considering the limitations due to the limited scientific facilities and the restrictions imposed by World War II, the work of Irma Pierpaoli provided a remarkably good representation of the macroalgal flora of the area of Ancona. The long-term changes unraveled by the comparison of the two herbaria are probably common to many other port areas in the Mediterranean and suggest the importance of continued monitoring in the future. This study represents an additional piece of evidence supporting the great value of macroalgal herbaria for the study of temporal variations in coastal communities.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/d16120741/s1, Figure S1: Maps of the port of Ancona at the time of Pierpaoli's collections (a) and an aerial image of the same area at present (obtained from Google Earth); Table S1: List of sites and indications of locations marked by Irma Pierpaoli for specimens deposited in her herbarium; Table S2: List of sites for the specimens deposited in the ANC ALG herbarium; Table S3: List of the specimens from the study area conserved in the Pierpaoli herbarium (sheet 1 green algae, sheet 2 red algae, and sheet 3 brown algae); Table S4: List of the specimens of the ANC ALG herbarium; Table S5: Comparison of collections in Pierpaoli and ANC ALG herbaria; Table S6: List of species reported by Pierpaoli (1941, 1946) for which no specimens in her herbarium were found; Table S7: List of the species documented from the area of Passetto di Ancona for which specimens are deposited in the Pierpaoli and ANC ALG herbaria.

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