Review

Ecological Function of Phenolic Compounds from Mediterranean Fucoid Algae and Seagrasses: An Overview on the Genus Cystoseira sensu lato and Posidonia oceanica (L.) Delile

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Abstract: Biodiversity is undergoing rapid and worrying changes, partially driven by anthropogenic activities. Human impacts and climate change (e.g., increasing temperature and ocean acidification), which act at different spatial scales, represent the most serious threats to biodiversity and ecosystem structure and function. In the Mediterranean Sea, complex systems such as fucoid algae and seagrasses, characterized by a high associated biodiversity, are regularly exposed to natural and anthropogenic pressures. These systems, particularly sensitive to a variety of stressors, evolved several physiological and biochemical traits as a response to the different pressures which they are subjected to. For instance, they produce a huge quantity of secondary metabolites such as phenolic compounds, to adapt to different environmental stressors and to defend themselves from biological pressures. These natural products are receiving increasing attention due to their possible applications in a wide range of industrial sectors. In this paper we provide an overview on the ecological role of phenolic compounds from the genus Cystoseira sensu lato and Posidonia oceanica (L.) Delile, also highlighting their potential use as ecological biomarkers.

Keywords: phenolic compounds; ecological role; Cystoseira sensu lato; Posidonia oceanica; biomarkers; Mediterranean Sea

1. Introduction

Marine algae and seagrasses produce secondary metabolites to adapt and acclimate to environmental stressors, but also to defend themselves from biological pressures such as competitors, pathogens, grazers and epiphytes [1–7]. The production of these metabolites is known to vary with environmental conditions (e.g., light, nutrient, and temperature), but also with geographical distribution and biological status (e.g., life cycle) [8–11].

Among secondary metabolites, phenolic compounds, carbon-based metabolites ranging from simple molecules, such as free and ester-bound phenolic acid, to more complex polymers such as condensed tannins and lignins, are commonly produced by algae and seagrasses for structural, protective and ecological purposes [4–6,12–17]. For instance, algae and plants living in saltworks produce and accumulate polyphenols as defense strategies to cope with these extreme environmental conditions [18].
In the Mediterranean Sea, which is a true hotspot of biodiversity [19–21], large, brown, fucoid algae of the genus *Cystoseira sensu lato* (Fucales, Phaeophyceae), according to Orellana et al. [22], and seagrasses like *Posidonia oceanica* (L.) Delile (Posidoniaceae, Alismatales) (Figure 1), supporting high biodiversity and providing essential ecological goods and services, are valuable, productive and unique habitats [19,21,23,24]. Since the Mediterranean basin is also one of the main climate (e.g., increases of temperatures and CO$_2$) change “hot-spots” [25] and impacted sea, these valuable communities are significantly threatened by all these pressures which are predicted to increase in the future [24,26,27].

![Figure 1. Cystoseira sensu lato community (A), Posidonia oceanica meadow (B).](image)

Fucoid algae and seagrasses, particularly sensitive to a variety of stressors, evolved several physiological and biochemical traits as response to the different pressures they are subjected. For instance, they produce a huge quantity of phenolic compounds playing multiple roles [17,28–33]. Since these compounds also have several biological properties, they are receiving increasing attention due to their applications in a wide range of pharmaceutical (e.g., anticancer, antimicrobial, antifungal or anti-inflammatory), medical and industrial sectors [18,34–36]. Tannins are considered to be one of the most broadly distributed phenolic compound types, and among them, phlorotannins (halogenated or sulphated oligomers or polymers of phloroglucinol—1,3,5-trihydroxybenzene, Figure 2A) constitute a heterogeneous group exclusively present in brown algae, such as fucoid algae, presenting chemical structures not found in terrestrial plants [13,37–40]. Seagrasses such as *P. oceanica* are also rich in phenolic compounds, as testified by the presence of “tannin cells” [1,2,41–43].

Since these systems are susceptible to human pressures, their health status is currently considered as an indicator of the environmental quality of the coastal zone. Therefore, understanding the mechanisms these valuable communities use to respond to different stressors is crucial to predict their responses to future climate scenarios. The aim of this paper is to provide an overview on the ecological functions of phenolic compounds from the fucoid algae of the genus *Cystoseira sensu lato* and seagrasses like *P. oceanica*, also evaluating their potential use as ecological biomarkers.

The search for relevant literature, updated up to summer 2019, was carried out using standard electronic databases (Google Scholar, Web of Science, Scopus and Pub Med). The research criteria were based upon the following list of keywords: “Fucales”, “fucoid algae”, “Cystoseira”, “seagrasses”, “Posidonia oceanica”, “phenol”, “metabolites”, “tannin”, “function”, “role”, “ecological”, “indicator”, “markers”, “Mediterranean Sea”.

2. Genus *Cystoseira sensu lato*

In the Mediterranean Sea, fucoid algae are important canopy-forming algae on a rocky substrata from exposed to sheltered conditions and from the intertidal zone to more than 50 m depth [44–48].
Recently, Orellana et al. [22] revised the genus *Cystoseira* sensu lato and recognized three different genera: *Cystoseira* sensu stricto, *Carpodesmia* Greville, gen. emend., *Treptacantha* Kützing, gen. emend.

These communities provide food, habitat and shelter for a large number of marine organisms, and are therefore considered biological “formers” of habitat structure [49–51].

For these reasons these algae are listed as “of community interest” according to the European Union’s Habitat Directive (92/43/EEC) [52]. Moreover, they are under surveillance by the IUCN (International Union for Conservation of Nature), the Berne Convention (Council of Europe, 1979) [53] and the Barcelona Convention (UNEP/MAP, 2009) [54]. Since these algae are particularly sensitive to variations of environmental factors [24,55,56], the Water Framework Directive (WFD, 2000/60/EC) [57] and the Marine Strategy Framework Directive (MSFD, 2008/56/EC) [58] considered them as reliable indicators of environmental quality in Mediterranean coastal waters [59,60]. Human pressures are increasingly limiting their distribution, and most of these algae are currently experiencing a severe decline in many Mediterranean regions [26,55,61–63].

2.1. Phlorotannins

Phlorotannins, exclusively present in brown algae, are biosynthesized through the acetate-malonate pathway in a Golgi apparatus and show chemical structures not found in plants [37,64]. They are mainly stored in special vesicles (physodes) within the cells. In this form they are soluble, whereas only a small amount is part of the cell wall, so phlorotannins have mainly a secondary role [4,39]. They have a wide range of molecular sizes (from 126 Da to 650 kDa) [10,13,65], and on the base of the polymerization patterns of the phloroglucinol subunits, can be divided into four distinct groups (Figure 2B–E): fuhalols and phloroethols (with ether linkages); fucols (with a phenyl linkage); fucophloroethols (with ether and phenyl linkages); eckols and carmalols (with dibenzodioxin linkages) [66,67]. Their production does not stop as the thallus gets older, but it decreases as a consequence of the thallus thickening and the decrease in photosynthesis [39]; phlorotannins also undergo turnover or degradation, and are released directly via exudation.

Fucoid algae are particularly rich in phlorotannins, that constitute up to 30% of the dry weight (DW) [13,68–70]. For different species belonging to the genus *Cystoseira sensu lato*, phlorotannins and phenolic compounds have been already isolated and identified [71]. Phlorotannins are involved in cell-wall hardening [72], function as a defense against herbivory, or for algae living in the intertidal littoral zone, as adaptation to wave exposure and periodical desiccation. They are released after the cells are damaged (as a consequence of grazing or tissue erosion). Moreover, they have a wide array of cellular (adhesion of zygotes to substrate, protection of the zygotes, wound-sealing and wound-healing) and ecological roles [4,10,39].

Phlorotannins also show antimicrobial, cytotoxic, antioxidant and antitumoral activities, and since they have up to eight interconnected rings, they are also more potent free radical scavengers than polyphenols derived from terrestrial plants [4,18,34,35,71,73–75]. However, since phlorotannins present a high degree of isomerization, it may be difficult to link biological activity to a particular isomer [76].
2.2. Ecological Role of Phlorotannins

Since phlorotannins vary in relation to intrinsic and extrinsic factors, their variability is particularly high [4]. But only soluble phlorotannins change with environmental conditions, as those present in the cell wall seem to be relatively stable to environmental variations [39]. Phlorotannins vary within and among species, in relation to the growth rate, the thallus morphology [4,10,34,37,77,78] (Figure 3) or age; for instance, adult thalli present longer and more complex forms, more difficult to degrade or release, than young thalli [39]. The content of soluble and cell-wall-bound phlorotannins also change within a single plant and among individuals within the population.

![Chemical structures of phlorotannins](image)

**Figure 2.** Chemical structures of phloroglucinol (A), eckol (left) and triphlorethohydroxycarmalol (right) (B), fucophloroethol (C), fucodiphloroethol (D), trifuhalol (E) (A, B, E modified from [4]; C, D modified from [71]).

![Total phenolic content](image)

**Figure 3.** Variations of total phenolic content (TPC) in four Mediterranean brown algae. Bars represent mean ± standard deviation (n = 5) (from [78]).
Phlorotannins are affected by seasons, light, depth, temperature and nutrient concentration [8, 11,28,68,69,79–81]. They have spatial variations or vary in accordance to biotic factors [10,34,37,77]. For instance, Le Lann et al. [69] found that during the reproductive period Sargassaceae produce phenolic compounds, possibly as a chemical protection against grazers or epiphytes or against UV radiation. In Carpodesmia amentacea (C. Agardh), Orellana and Sansón (synonym Cystoseira amentacea (C. Agardh) Bory), a significant negative correlation of phenolic content with seawater temperature, was found [81]. Latitudinal differences in phlorotannin content have also been observed, with a phlorotannin production higher in species from high latitudes [37,82–84]. In general, the intensity of pressures (grazing, epiphytes) increases with decreasing latitude [65,85].

The content of these compounds varies seasonally, with maximal contents exhibited in summer [8,80], in winter [86], in winter and spring [81] or from late summer to the middle winter [37]. In C. amentacea, differences between years were also observed [80,81] (Figure 4). According to Mannino et al. [80], C. amentacea showed the lowest value in winter when only the main axes are present, whereas in spring, when new branches, more vulnerable, are sprouted, they significantly increased, reaching the highest value in summer, when long primary branches with numerous leaf-like structures were present. This finding is in accordance with Van Alstyne et al. [87] who found an intra-thallus distribution of these compounds, usually more concentrated in the meristematic zones, i.e., the distal extremities.

![Figure 4. Carpodesmia amentacea: seasonal variations of the TPC and sea water temperature. Bars represent mean ± standard deviation (n = 5) (from [81]).](image)

In Cystoseira baccata (S.G. Gmelin) P.C. Silva, an increase of these compounds from spring to summer was observed [69], and in summer an increase of large compounds (especially those with sizes between 2000 and 5000 Da), acting as photoprotectants compounds, was found. Indeed, large molecules have putative defense and protection roles as proved by antioxidant activity values higher than those of small compounds [88,89]. High irradiances during low tides or the high transparency in the coastal waters, which allows high penetration of both photosynthetically active radiation (PAR) and ultraviolet radiation (UVR), can produce negative biological effects such as photoinhibition or DNA damage. Under high PAR and UVR, photoprotection systems are activated, for instance UV screen compounds (phlorotannins) with a strong antioxidant activity [38,79,90].

Carpodesmia tamariscifolia (Hudson) Orellana and Sansón (synonym Cystoseira tamariscifolia (Hudson) Papenfuss) has higher rich physodes, mainly located in cortical cells, which provides
the photoprotection of cytoplasmic organelles [91]. In \textit{C. tamariscifolia}, these compounds are accumulated mainly at the apices [9], the part of the thalli with the highest light exposure. The rapid synthesis and turnover time of these compounds observed in \textit{C. tamariscifolia} might serve as photoprotective mechanisms against high irradiances [8]. \textit{Cystoseira tamariscifolia} acclimates to high UVB by up-regulating UV screen substances that also act as antioxidants [92]. Their content and the antioxidant activity were highest in \textit{C. tamariscifolia} in ultraoligotrophic waters with high transparency, suggesting an increase of the photoprotection capacity related to the photoacclimation to high irradiance, thus preventing the photodamage. Therefore, the alga minimizes damage from high irradiance, not only by the downregulating process in photosystems, but also by the production of UV photoprotectors and antioxidant compounds [60,92]. The release of these compounds from \textit{C. tamariscifolia} thalli at noon has been suggested to be a photoprotective mechanism under high irradiance conditions [8,11]. The combination of thallus thickness, phenolic compounds (and their positive response to temperature increase), antioxidant activity and the reduction in UV penetration by the release of these compounds, might give \textit{C. tamariscifolia} an advantage over other algae in high light conditions.

A decrease in soluble phlorotannins with high nutrient levels was observed, and such effect was often explained by the fact that nutrient enrichment commonly leads to an increase in growth [93], which, in turn, causes shading followed by the decrease in soluble phlorotannin production. But in \textit{C. tamariscifolia} a higher content when nitrate levels were high has been observed [11]. In \textit{C. tamariscifolia} increased irradiance in spring enhanced productivity, antioxidant activity and the production of photoprotective compounds, but in summer nutrient depletion due to thermal stratification of coastal waters reduces photosynthetic activity and the photoprotective capacity of the alga [60]. In spring, the alga uses photosynthetic energy both for the accumulation of carbon compounds to store energy and to build up photoprotective compounds.

Phlorotannins also function as a chelating agent of toxic heavy metal ions [94]. Their increase in the cell-wall, together with the increase in their exudation in the surrounding water, could prevent the copper from entering and damaging the photosynthetic apparatus allowing the seaweeds to tolerate high copper contamination [95].

Ocean acidification is expected to increase algal productivity, boost antioxidant activity and increase production of photoprotective compounds [96]. Under elevated pCO$_2$ conditions an increase in the biomass of \textit{C. tamariscifolia} has been observed, but only at optimal temperature conditions and also if nutrients were sufficiently available. In \textit{Cystoseira compressa} (Esper) Gerloff and Nizamuddin, these compounds increase under high pCO$_2$ levels independently from temperature, highlighting that the ocean acidification can positively affect algal photoprotection [97].

The bathymetric level, acting on the herbivore pressure, affects their content. Differences in bathymetric level also imply differences in water movement that have direct effects on herbivore pressure. Grazing pressure is generally lowest in wave-exposed shores where the feeding ability of consumers is limited to calm periods [98]. Therefore, in the infralittoral fringe a greater water movement makes feeding on algae more difficult than in the constantly submerged zones. Indeed, in \textit{C. amentacea} inhabiting the infralittoral fringe, a lower content than that found in infralittoral species such as \textit{Dictyopteris polypodioides} (A.P. De Candolle) J.V. Lamouroux, suggests that the last one invests more resources in defensive metabolites due to a higher grazer pressure [78] (Figure 4). Phlorotannins also induce resistance to grazing [99]. Induced resistance (increase of phlorotannins) decreases grazing pressure by deterring herbivores (grazed algae avoids future clipping from grazers which prefer ungrazed algae), as well as impairing their performance [100]. Increased excretion of phlorotannins into the water as a consequence of the feeding process was also observed [39]. A fraction of phlorotannins is released as a consequence of stress conditions such as grazing, but another fraction is regularly released into the surrounding water [39,101,102].

Reductions in their content might be related to phlorotannin exudation but also to polymerization of these molecules into more complex forms, difficult to degrade [39]. Herbivore pressure varies
also in relation to thallus morphology. Sheet algae (*D. polypodioides*), resulting more palatable than leathery (*C. amentacea*) or crustose ones [103,104], are expected to produce a higher amount of these compounds [80].

The bathymetric level, however, also affects exposure to solar radiation. For *Cystoseira sensu latu* species inhabiting the infralittoral fringe and subjected to regular even if incomplete emersion of the thalli, which implies a direct exposure to solar radiation, a higher content of these compounds as compared with those of species inhabiting the continually submerged zone, is expected [105]. Instead, in *C. amentacea* and in *C. compressa*, inhabiting the infralittoral fringe, a lower value than *D. polypodioides*, inhabiting the continually submerged zone, was found [78]. Connan et al. [79] reported a higher content of these compounds in brown algae growing at mid-tide level compared to that of species inhabiting either the low-tide level or the upper mid-littoral fringe. The authors hypothesized that other mechanisms of photoprotection exist, such as carotenoids, which might act together with these compounds. Thus, the stressing condition of the emersion phases does not necessarily result in an increase of these compounds. Moreover, Le Lann et al. [70] found a decrease in the content of small molecular weight (<2000 Da) in relation to an increase within the depth, hypothesizing a possible photoprotectant role for small compounds.

### 3. Posidonia oceanica

Seagrasses, flowering and rhizomatous plants exclusively living in marine habitats, are key coastal species which provide valuable ecological services, oxygen production, sinks for CO2 emissions, sediment stabilization and coastal protection from erosion [106,107]. They support valuable ecosystems, characterized by high productivity and associated biodiversity, having a potentially significant role also in climate change mitigation [108]. Seagrasses, belonging to the monocotyledonous order Alismatales and assigned to four different families Cymodoceaceae, Hydrocharitaceae, Posidoniaceae, and Zosteraceae [109,110], form worldwide extensive meadows in coastal systems except for Antarctica [109]. Seagrasses live in a wide geographical range and are limited to the coastal continental shelf (0-45 m). Five of the total number of seagrass species are found in the Mediterranean Sea, precisely *P. oceanica*, *Cymodocea nodosa* (Ucria) Ascherson, *Zostera marina* L., *Z. noltii* Hornemann and *Halophila stipulacea* (Forsskål) Ascherson (non-indigenous species).

*Posidonia oceanica*, the dominant endemic seagrass species in the Mediterranean Sea, plays a crucial ecological role in the coastal zones of tropical and temperate seas, providing food and habitat for a large number of associated organisms [111,112]. *Posidonia oceanica*, a plant with a slow growth rate and low genetic diversity, forms meadows which represent, on soft bottoms, the ecosystems with the greatest primary productivity, part of which is exported to neighboring systems. *Posidonia oceanica* can grow in extraordinarily diverse environmental conditions [113], thanks to physiological and morphological adaptations which sometimes only endemic species possess. *Posidonia oceanica* was included by the European Union’s Habitat Directive (92/43/CEE) among the habitats of priority interest [114], and is protected under the Bern and the Barcelona Conventions, and other legislations at a national level. Due to its high sensitivity, *P. oceanica* is considered as a biological indicator of environmental quality [115]. Recently, the MFSD (2008/56/EC) [58] selected *P. oceanica* as representative species of the angiosperm quality elements for the Mediterranean marine environment. Despite all that, *P. oceanica* habitats are experiencing decline or loss throughout the Mediterranean, often due to synergies among multiple stressors, mainly of anthropogenic origin, which are particularly threatening slow-growing species, such as seagrasses [27,116,117].

#### 3.1. Phenolic Compounds

In seagrasses, phenolic compounds are produced via the Shikimic Acid Pathway in specialized cells called “tannin cells” [42,118]. Small molecules are able to rapidly diffuse across cell membranes to reach intracellular sites or remain as pigments in the senescent phase of the cell, whereas several larger
polyphenols, biosynthesized in situ from smaller polyphenols to non-hydrolyzable tannins, remain not detected in the plant matrix [119].

These compounds are produced to function as defense mechanisms, in response to stress conditions, such as abiotic factors, non-indigenous species (NIS), competitors, predators and pathogens [2]. Before the appearance of damage, *P. oceanica* can activate self-protection mechanisms, involving, for instance, phenolic compounds [29,120]. Polyphenols act through the release and suppression of growth hormones such as auxin, as UV screens to protect against ionizing radiation and to provide coloration (plant pigments), as a deterrent to herbivores, in the prevention of microbial infections (phytoalexins), as signaling molecules in ripening and in other growth processes [121]. Polyphenols also have a significant binding affinity for proteins, thus leading to the formation of soluble and insoluble protein–phenolic complexes [122], which influence the accessibility and activity of phenolic compounds [123].

Currently, various phenolic compounds were identified in *P. oceanica* [1,2,4,12,24,125]. In *P. oceanica* leaves, tannins account for ca. 5–10% [15], whereas 2% of the dry weight of leaves corresponds to free or ester-bound phenols [2,3,126]. The remaining amount of phenol is poorly understood. Agostini et al. [2] identified a total of 23 compounds in *P. oceanica*. Of these, 20 are phenolic and cinnamic acid derivatives. Leaves are rich in chicoric, caftaric and gentisic acids, but other phenols, such as the aldehyde vanillin, and *p*-coumaric, ferulic, caffeic and cinnamic acids, and phenol derivatives (e.g., phloroglucinol), benzoic acid derivatives, calchones and proanthocyanidins are present [6,127] (Figure 5). In leaves, different compounds resulted as main components, for instance, ferulic acid [2,125] or *p*-hydroxy benzoic acid [124] or gentisic acid [14].

![Figure 5. Phenolic compounds in Posidonia oceanica.](image)

Even though seagrasses are supported by the hydraulic forces of the water body, lignin was detected in several tissue types and several cells, and variations of the lignin content in relation to the morphotype and life style of the seagrass were observed [128,129]. Lignin is particularly abundant in *P. oceanica*, with a higher content in roots and rhizomes than in leaves [130]. The lignin, a typical constituent of angiosperm ancestors, would contribute to the longevity of the tissue by protecting it against microbial attack, even if its deposition is restricted to tissues that show limited growth.
3.2. Ecological Role of Phenolic Compounds

Among the Mediterranean seagrasses, *P. oceanica* is the most thoroughly investigated from the phytochemical point of view [6]. Several studies analyzed the role that phenolic compounds have in the response of *P. oceanica* to different environmental pressures: e.g., turbidity and pollution, ocean acidification, competition, pathogen infection and overgrazing [2,15,29,30,42,120,125,131–133]. For instance, high phenol concentrations were found in *P. oceanica* leaves when exposed to contamination by metals and proximity to intensive fish aquaculture [42,134].

A negative relation between grazing pressure and phenols was found [135]; indeed, tannins are known to reduce palatability and increase the toxicity to herbivores [10]. The increase of phenols in response to grazing was also explained as a defense mechanism against infection, since grazing provides an opening for pathogenic organisms [136]. Moreover, Dumay et al. [42] observed that in *P. oceanica* the number of the tannin cells changed in response to herbivores. Furthermore, Cozza et al. [137] observed a variation in the typology of the phenolic compounds in tannin cells, in response to environmental injuries. Seagrasses produce several types of phenolic compounds (e.g., phenolic acids, condensed tannins and lignins), thus a specific class of phenolic substances could be involved in the response mechanisms of *P. oceanica*. Phenolic compounds also act as metal chelators, providing an internal detoxification mechanism against heavy metals [2] and key allelopathic agents [42]. *Posidonia oceanica* is able to accumulate significant amounts of heavy metals, and this accumulation is responsible for a reduction in total phenolic compound content [133]. Moreover, they are also involved in the cell-wall hardening [42].

The production of phenolic compounds changes in relation to species, growth phase and environmental conditions, and it is also genetically controlled. Haznedaroglu and Zeybek [14], for instance, found in young leaves a higher quantity of chicoric acid. In the marine realm, the genetic variability and phenotypic plasticity of the species confer an adaptive mechanism to environmental disturbances by producing, for instance, secondary metabolites, which allow for an efficient plant acclimation [16,29,138]. The changes in phenol metabolism could be a result of the adaptation to the marine environment [129]. *Posidonia oceanica*, in the presence of competitors such as *Caulerpa taxifolia* (M. Vahl) C. Agardh, apparently invests in defense rather than growth [15,42,125]. It would favor the synthesis of secondary metabolites through an increase in the number of tannin cells, thus supporting the idea that phenolic compounds are involved in defense against pathogens and allelopathy [15,42,118].

Since the changes in phenolic compounds represent a response to environmental stressors, they can be used to screen the *P. oceanica* health status during the regular monitoring programs carried out for management purposes [16,17,113,116,132,139,140]. Two promising tools (easy, rapid and inexpensive): phenolic compounds in rhizome and Random Amplified Polymorphic DNA (RAPD) markers in leaves, were recently proposed to monitor the health state of *P. oceanica* meadows [17,116,132]. In fact, phenolic compounds represent a generic response to different environmental stress related not only to extrinsic (seasons) and intrinsic (metabolism) factors, but also to the genetic variation of the plants. RAPD markers have been used to assess the pattern of genetic diversity and the genetic structure for the conservation of endangered plants, revealing a decreased genetic diversity in *P. oceanica* meadows along an anthropogenic disturbance gradient, both at small, e.g., within a meadow, and at Mediterranean scale [116].

Recently, the conservation status of the *P. oceanica* meadow at two protected areas, S. Marinella (SIC “Situ of Community Importance”, Central Tyrrhenian Sea), situated 100 km far from Rome (Italy), and Monterosso a Mare (Northern Tyrrhenian Sea), were evaluated through a biochemical/genetic approach: phenolic compounds and Random Amplified Polymorphic DNA (RAPD) markers [17,29,132]. A high quantity of polyphenols was found at Santa Marinella, where *P. oceanica* was ranked as a disturbed one. This low ranking was confirmed by the mean phenol content, quite high and scattered, and the low genetic variability, with a very high similarity of specimens at a local scale [132,140].
Moreover, in the Talamone meadow (Florence, Italy), judged as “well preserved”, phenol content was lower than in Santa Marinella plants [132]. At Monterosso a Mare, the mean total content showed a significant seasonal variation, with higher concentration in summer than in winter [17]. The significant phenol content increase in summer could be related to disturbance events. Therefore, in P. oceanica the variation in the production of polyphenols within the meadow would reflect not only the impact of multiple anthropogenic stressors (e.g., vessel traffic, pollution), but also the variation of abiotic and biotic factors during the seasons [17,29,132]. These differences account for changes in metabolic/physiological pathways as an adaptation to stress and extrinsic factors like seasonal variations in temperature. In leaves, total phenol concentration also varies seasonally due to the leaves’ short lifespan and with depth [42]. On the contrary, levels of synthesis and accumulation of phenolic compounds were more stable in rhizomes [29]. A higher concentration of phenols in the intermediate leaves of P. oceanica was observed during winter, when growth conditions are limiting [15,42,120,133,141]. Consequently, in P. oceanica the growth process seems to dominate the defense process when the environmental factors are favorable for growth [142,143].

Changes in CO2 concentrations modify the capacity of plants to resist or tolerate herbivory [144]. According to the resource availability hypothesis (RAH, [145]), seagrasses growing under elevated nutrient availability will reduce the production of chemical defenses such as phenols [146,147]. Since CO2 can greatly limit primary production in seagrasses [148,149], under high CO2 scenarios a decrease in phenolics would be expected, following RAH. In P. oceanica plants, a decrease [30,150,151], as well as no changes [147] in phenolic compounds, have been reported, with a consequent increased feeding of a wide range of consumers, including fishes and sea urchins [30,150–152]. Under high CO2, a decrease in phenolic compounds was also observed in the seeds of P. oceanica, which were bigger, with higher carbon content and more stored sucrose [152]. Having more resources (i.e., CO2) available, they invest less on defense, favoring the storage of more rapidly available carbon-based compounds such as sucrose [153].

4. Conclusions

The Mediterranean is currently experiencing decline and/or loss in the number of species and a deterioration of habitats, mainly related to the increase of human activities and global change [19,21,154–157]. The high sensitivity of valuable and productive habitats, such as the genus Cystoseira sensu latu and P. oceanica meadow, makes them particularly vulnerable and susceptible to human pressures. Since human stressors are expected to increase in the coming decades, a strong effort at the global scale is required in order to establish and/or implement effective conservation plans for these sensitive and fragile ecosystems [158]. To support the sustainable management of these ecosystems, continuous monitoring of their health status and their changing over time is essential. Since these systems are able to provide an early warning of suffering or decline and deteriorating environmental quality [23,159,160], they can act as a benchmark for monitoring the environmental health of marine systems [17].

Biomonitoring is an expensive and time-consuming activity, and thus the appropriate choice of indicators is essential. Consequently, the identification of effective and sensitive diagnostic tools and measurable descriptors suitable for monitoring the health status of marine systems is a critical issue. Since the synthesis and storage of phenolic compounds in Cystoseira sensu latu and P. oceanica change in response to variations of several abiotic and biotic factors, they could be considered as the potential valuable markers of stress conditions. The punctual response of phenols to several stress conditions seems to support its feasibility as an early warning indicator. Moreover, the inexpensiveness of these approaches makes it easy to introduce them in meadows monitoring.

Recently, biochemical and genetic approaches have been proposed as suitable and convenient biomarkers of the health status of P. oceanica and/or other seagrasses. Indeed, they allow us to depict plant physiological stress and environmental disturbance as well as traditional methods can [17].
High levels of total phenols, together with the low genetic variability (as RAPD markers), identified endangered *P. oceanica* meadows, perfectly in line with the results of traditional methods (density, leaf biometry).

However, even though in *P. oceanica* phenolic compounds changes which are in response to environmental pressures are well known [15,29,30], yet they are still scarcely considered as environmental biomarkers in monitoring plans.

Even if additional researches are necessary to define thresholds for classifying the different level of perturbation, the obtained results open interesting perspectives on the application of the phenol content in *Cystoseira sensu latu* and *P. oceanica* as possible biomarkers of environmental quality. In monitoring surveys, the assessment of health marine ecosystems should make provision for multidisciplinary investigations [161,162] and multiparametric approaches, including physiological biomarkers, biomarkers of general stress and more specific biomarkers [131], but also molecular ones [16,27,140,163].

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