Modelling antifouling compounds of macroalgal holobionts in current and future pH conditions

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ABSTRACT

Marine macroalgae are important ecosystem engineers in marine coastal habitats. Macroalgae can be negatively impacted through excessive colonization by harmful bacteria, fungi, microalgae, and macro-colonisers and thus employ a range of chemical compounds to minimize such colonization. Recent research suggests that environmental pH conditions potentially impact the functionality of such chemical compounds. Here we predict if and how naturally fluctuating pH conditions and future conditions caused by ocean acidification will affect macroalgal (antifouling) compounds and thereby potentially alter the chemical defence mediated by these compounds. We defined the relevant ecological pH range, analysed and scored the pH-sensitivity of compounds with antifouling functions based on their modelled chemical properties before assessing their distribution across the phylogenetic macroalgal groups and the proportion of sensitive compounds for each investigated function. For some key compounds, we also predicted in detail how the associated ecological function may develop across the pH range. The majority of compounds was unaffected by pH, but compounds containing phenolic and amine groups were found to be particularly sensitive to pH. Future pH changes due to predicted average open ocean acidification pH were found to have little effect. Compounds from *Rhodophyta* were mainly pH-stable. However, key algal species amongst *Phaeophyceae* and *Chlorophyta* were found to rely on highly pH-sensitive compounds for their chemical defence against harmful bacteria, microalgae, fungi, and biofouling by macro-organisms. All quorum sensing disruptive compounds were found the be unaffected by pH, but the other ecological functions were all conveyed in part by pH-sensitive compounds. For some ecological keystone species, all of their compounds mediating defence functions were found to be pH-sensitive based on our calculations, which may not only affect the health and fitness of the host alga resulting in host breakdown but also alter the associated ecological interactions of the macroalgal holobiont with micro and macrocolonisers, eventually causing ecosystem restructuring and the functions (e.g. habitat provision) provided by macroalgal hosts. Our study investigates a question of fundamental importance because environments with fluctuating or changing pH are common and apply not only to coastal marine habitats and estuaries but also to freshwater environments or terrestrial systems that are subject to acid rain. Hence, whilst warranting experimental validation, this investigation with macroalgae as model organisms can serve as a basis for future investigations in other aquatic or even terrestrial systems.

Keywords: Macroalgae, micro, and macro-colonizers, ocean acidification, climate change, antifouling

INTRODUCTION

Marine macroalgae (also termed seaweeds) are diverse photosynthetic organisms present throughout marine ecosystems. As ecosystem engineers, macroalgae provide a suite of ecologically valuable functions such as nutrient cycling, carbon sequestration, sediment stabilization, and habitat provision to a range of dependent marine flora and fauna (Chung et al. 2011; Costanza et al. 1997). Hence, macroalgae support a variety of productive and diverse coastal marine ecosystems (Kumar et al. 2016). Macroalgae surfaces are colonized by complex microbial communities, which form a unified functional entity or 'holobiont' (Egan et al. 2013) with functions related to host health, development, and defence against micro and macro-organisms (Egan et al. 2014). This biofilm-like epimicrobiome, known as the 'second skin', is mainly dominated by epibacteria (Wahl et al. 2012). Like their unicellular ancestors, macroalgae-bacteria associations range from beneficial (mutualistic), to neutral (commensal), facultative, obligate, and harmful (pathogenic) interactions (Relman 2008). Positive macroalgae–bacterial ('friendly') interactions include phytohormone production, morphogenesis of macroalgae triggered by bacterial compounds, specific antibiotic activities affecting epibionts, and elicitation of oxidative burst mechanisms (Weinberger 2007; Wichard 2015; Saha & Weinberger 2019). Some bacteria can prevent biofouling by micro- (e.g. diatoms) or macrofoulers (e.g. barnacles, mussels) or pathogen invasion and extend the defence mechanisms of the macroalgae itself by producing bioactive secondary metabolites (Egan et al. 2008; Nasrolahi et al. 2012; Wahl et al. 2012). Deleterious macroalgae–bacterial ('unfriendly') interactions can induce or generate algal diseases, affect the host's fouling sensitivity (Wahl 2008), or its susceptibility to grazers, which may, in turn, increase pathogenic infection through grazing wounds (Wahl et al. 1997).

Bacterial colonization or microfouling is not random but highly controlled in many macroalgae via chemically-mediated interactions (reviewed by Goecke et al. 2010; Saha et al. 2018). To deter or minimize settlement, growth, and biofilm formation by bacteria, macroalgae can influence bacterial metabolism and quorum sensing and produce antibiotic compounds (Dobretsov et al. 2006). Associations between these two cross-kingdoms are dependent on infochemicals (information carrying chemicals) (Saha et al. 2019). Studies directly investigating the influence of surface-associated metabolites (or compounds) on bacterial surface colonization have demonstrated how specific macroalgal extracts have a marked effect on bacterial biofilm formation and community composition under both laboratory and field conditions (Lachnit et al. 2010; Sneed & Pohnert 2011). Recently, it was demonstrated that, like the human gut and the rhizosphere of terrestrial plants, macroalgae can also specifically engage in chemically mediated microbial 'gardening', recruiting beneficial microbes while deterring settlement of pathogenic bacteria (Saha & Weinberger 2019). Chemical compounds that act antibacterial (AB), cause quorum sensing disruption (QSD) to disrupt bacterial communication, or compounds with antibiofilm functioning (ABF) play a crucial role in these interactions.

Along with bacteria, macroalgae get also colonized by other microcolonisers like fungi, microalgae, and macrocolonisers like barnacles, mussels, and other invertebrates. Uncontrolled colonization can often have a multitude of mostly detrimental consequences on the algal host: increased weight and friction, impeded trans-epidermal exchanges, altered colour, smell, and contour. These proximate changes to the host due to epibiosis (or fouling) may lead to a loss of buoyancy, an impediment of motility, a hindrance to mating, or a substantial shift of interactions among species (Wahl et al. 2012). Thus, macroalgal holobionts are known to deter or minimize colonization by other micro- and macrofoulers *via* chemical compounds or metabolites with antimicroalgal (AA), antifungal, and antimacrofouling (AMF) effects (reviewed by da Gama et al. 2014). These compounds have a substantial impact on interspecific and intraspecific communication, and population- and ecosystem-level interactions (Saha et al. 2019).

Communication *via* these metabolites is mediated mainly through the surface of macroalgae - the primary physiological and ecological interface with the environment (Wahl 2008). Apart from respiration, absorption of energetic radiance, and exchange of nutrients, this active functional interface is where defensive (repulsive) or stimulating (attractive) molecules are released, localized, and potentially diffused into the surroundings, depending on the chemical nature of the molecules. Thus, this delicate interface is the chemical 'playground' for the attraction of allies using pro-fouling compounds and employing anti-fouling compounds against enemies. However, epibiosis is not always restricted to the surface of the alga and certain epibionts are known to penetrate the cortex and outer medulla of macroalgae and thus interact with the intracellular macroalgal defence compounds (reviewed by da Gama et al. 2014). Apart from inhibiting or minimizing initial colonization, macroalgal chemical defence against colonizers can also act by enhancing their post-settlement mortality (Wikström & Pavia 2004). The location of all these interactions at or near the algal surface exposes the compounds to the physicochemical conditions, e.g. pH and temperature, in this surrounding (Fig. 1).

Rapid global scale (ocean warming, acidification) and regional scale (hypoxia, desalination) climate change will not only affect the physiology of plants and animals but may also modify their chemically-mediated interactions by altering the production, reception, and chemical characteristics of such compounds (Leduc et al. 2013). In 2110, average seawater temperatures are predicted to have increased by ~5°C, and the atmospheric carbon dioxide partial pressure (pCO_2) is expected to reach approximately 1000 ppm (Fifth IPCC report, IPCC, 2014; RCP 8.5). Ocean acidification (hereafter OA) will not only affect calcification (Beaufort *et al.*, 2011), but reduced pH can also affect the compounds themselves (Hardege et al. 2011). OA has been shown to impact chemical communication in marine animals by (a) changing the molecules' structure (Roggatz et al. 2016, 2019) or (b) reducing the ability of organisms to sense these compounds (Munday et al. 2009).

Compounds of biological origin often contain one or multiple functional chemical groups such as amines, thiols, or phenols that are sensitive to pH. These groups can be protonated or deprotonated (addition or removal of a proton) with changes in pH. The pH at which there are 50% of the molecules protonated and 50% remain unchanged is expressed by a group-specific acid dissociation constant (pK_a) (Po & Senozan, 2001), a useful indicator of a compound's pH-sensitivity. The pK_a constant also can be used to calculate the proportion of protonated, unprotonated, and/or deprotonated molecules in solution at any given pH (Po & Senozan, 2001). Different protonation states can differ significantly from each other as protonation alters the overall compound structure, its charge, and potentially the conformation (3D shape) of the molecule (Roggatz et al. 2016). The alteration of such key molecular properties can disrupt the messaging cascade; hence the de-/protonated form of the compound is rendered non-functional (Roggatz et al. 2016, 2019). A shift in the abundance of a protonation state can therefore also translate directly into an increase or decrease of the compound-associated ecological function.

Much of the previous work on the impact of OA on chemical communication of marine organisms has focused on the response of marine fauna, yet the primary producers of the oceans like macroalgae are equally, if not more, dependent on chemical communication as demonstrated above. Chemically mediated interactions of macroalgae can be responsible for structuring entire communities (Korpinen et al. 2007). However, how climate change stressors like OA can alter the protonation state of the signalling compounds, and thereby potentially the mediated ecological interactions, has not been given any attention to date (Schmidt & Saha 2020).

Thus, in the current study, we focus on modelling antifouling compounds and investigate if such compounds are sensitive to natural (Saderne et al. 2013) and future pH fluctuations, as these fluctuations are predicted to increase

significantly in future oceans (Landschuetzer et al. 2018). To do so we identify the chemical functional groups making them pH-sensitive. Therefore, we define the relevant range of natural pH fluctuations based on recently published literature, collect chemical information about the compounds' ionisation and calculate the likelihood of a change in protonation state abundance over the pH range in order to score the compounds' pH-sensitivity. We (a) further analyse the distribution of pH-sensitive antifouling compounds across phylogenetic groups with different ecological functions, and (b) evaluate the potential impact of future pH reductions on their ecological functioning.

MATERIALS & METHODS

Selection and compilation of known antifouling compounds

We selected studies that investigated the effects of macroalgae/macroalgal compounds on marine organisms from the Web of Science core collection for papers published between 2010 – 2018 and based on the recent review by Saha et al. (2018). We chose this range intending to provide a holistic overview of the functionality of such compounds under naturally fluctuating and future pH and to draw the attention of the research community to this unexplored aspect of algal chemical ecology. The following search conditions were used: TI = (antifouling OR fouling OR antibacterial OR antibiofilm OR antifungal OR antimicroalgal OR quorum sensing AND TS= (seaweed* OR macroalga* OR alga*) AND TS= (marine OR seawater) NOT TS = (lake OR freshwater). We included studies that tested the activity of macroalgal compounds against marine micro and macro-colonizers. Medical and industrial fouling investigations or studies testing non-marine organisms including bacteria were not used in our analysis.

Definition of current and future pH ranges

Average sea surface pH conditions may not always be representative, especially when looking at highly fluctuating environments such as coastal, estuarine, and tidal realms (Wahl et al. 2016). The correct identification of the naturally occurring pH range is therefore crucial when studying the impact of pH on chemical communication at the molecular chemical level. Hence, we conducted a thorough literature search for pH values measured in the close vicinity of macroalgae and combined our findings into a pH range relevant to this study of chemically-mediated macroalgal interactions with organisms on its surface.

Acid dissociation constants and calculation of protonation state abundance

We undertook an extensive literature search for each compound to identify known acid dissociation constants (pK_a) where possible. Due to most compounds being only recently identified, little chemical characterisation information was available. We, therefore, calculated the pK_a constants for those compounds where no literature values could be found using the Chemicalize Calculation web application (https://chemicalize.com/) by ChemAxon (ChemAxon Ltd., 2019). This application is also frequently used to estimate pK_as for chemicals in the pharmaceutical industry and informs a wide range of databases. The ChemAxon package has been found to predict pK_a constants to an accuracy of ±0.5 or better for functional groups of interest in this study (Settimo et al. 2013). The collected constants were then utilised to calculate and plot the abundance of different protonation states across the pH range.

Calculation of the protonation state abundance curves was based on the Henderson– Hasselbalch equation (for details see Po & Senozan 2001) and calculated in steps of 0.1 for pH values between 0 and 14. At pH 0 the [H^+] was set to 0 and the abundance of the fully protonated form was set to 1 (100%) as a reference.

Temperature affects acid dissociation constants, particular those of amine and phenolic functional groups. For every 10°C temperature increase, the respective pK_a constant is reduced by 0.1 to 0.2 units (Reijenga et al. 2007), leading to a shift in protonation state abundances by ± 3 to 5% for an average predicted temperature increase of +4°C (IPCC, 2014) and the compounds included in this study (data not shown). This impact is reflected in our scoring by defining broad categories with steps of 5% change or more (see below).

Scoring of compounds' pH-sensitivity and analysing the impact on their ecological functioning

Based on the obtained abundance curve for each compound, we calculated the change of each protonation state (in %) across the current and future pH range. Compounds with no change in protonation within these ranges were scored as 'insensitive/ pH-stable' and not affected by pH within the environmentally relevant range in this context. Compounds with a change in protonation were scored according to their sensitivity to pH in four categories as shown in Table 1. To assess the impact of future ocean conditions (based on RCP8.5 predictions (IPCC, 2014)), we compared the change in protonation state abundance between current and future pH ranges. This comparison reveals if a lower overall pH range due to ocean acidification affects the protonation pattern of the macroalgal compounds and hence alters their sensitivity.

To translate our numerical results into an ecological-functional context, we assumed that the protonation state dominating across the current pH range is the bioactive form and the molecular alteration caused by de-/protonation renders other protonation states non-functional, as it has been observed in previous studies (Roggatz et al. 2016, 2019). Significant changes to the abundance of a dominating protonation state can therefore be interpreted as a strong indication of a change to the compound's functioning.

RESULTS

List of the selected antifouling compounds, their structures, and biological functions

Table 2 lists all compounds used in his study with their respective biological source, compound name, known function(s), chemical structure (neutral), and reference. Most compounds are listed with trivial names; a list of the respective IUPAC names can be found in the Supplementary Information (S1). In total, our list contains 50 compounds, of which 23 were isolated from red macroalgae (*Rhodophyta*), 17 from brown macroalgae (*Phaeophyceae*), and 10 from green algae (*Chlorophyta*). The compounds serve a variety of functions: antibacterial activity (AB), antifungal activity (AF), antimicroalgal activity against diatoms, cyanobacteria, and red tide microalgae (AA), antimacrofouling including molluscs (AMF), quorum sensing disruption (QSD), and antibiofilm activity (ABF). 16 of the 50 compounds serve more than one function.

Environmentally relevant pH range

Recent research efforts have led to a detailed characterisation of natural pH fluctuations around macroalgae thalli and communities. Special attention has been paid to the impact of the diffusive boundary layer of water surrounding the macroalgae that creates a micro-environment. De Beer and Larkum (2001) measured that the coralline algae *Halimeda discoidea* experienced pH conditions ranging from 7.5 to 8.8 on the thallus surface, depending on the light conditions it was exposed to. Hurd et al. (2011) reported similar pH values fluctuating between pH 7.6 and 8.5 around the coralline seaweed *Sporolithon durum*. A comparable effect was also found for boundary layers around complex macroalgae assemblages (Cornwall et al. 2013). In stagnant water conditions, the pH in the vicinity of

Fucus vesiculosus was measured to range from pH 7.6 in the dark to pH 9.2 in the light (Wahl et al. 2016). Wahl and co-workers further established that while for bacterial biofilms and diatoms on the immediate surface of *F. vesiculosus* pH fluctuations can be large (exceeding ±1.0 pH units) and happen at a very short time scale of minutes, other organisms living in the vicinity of the macroalgae experience less strong fluctuations at longer time scales and the scale of fluctuation depends on the flow velocity of the surrounding water (Wahl et al. 2016).

Based on these findings we defined the environmentally relevant pH range influencing macroalgal compounds, which function close to the macroalgae thalli, to stretch from pH 7.6 to pH 9.2 in current conditions. Future pH conditions assumed to be on average 0.4 pH units lower than current sea surface pH (IPCC, 2014), would consequently shift the pH range to 7.2 to 8.8.

Sensitivity of compounds to pH fluctuations and relevant functional groups

Of the 50 compounds investigated, 72% (36 compounds) were unaffected by natural pH fluctuations. Of the remaining 28%, two compounds were mildly affected, eight were affected medium, two significantly, and two severely (Fig. 2, Table 3). Those compounds unaffected by the relevant pH range were either lacking ionisable groups entirely (eight compounds) or were found to possess functional groups with pK_as outside the affected range, mostly ketone and hydroxyl groups (=O, -OH) with pK_as above 12 and carboxylic groups (-COOH) with pK_as below 4.5. It could further be observed that many of the unaffected compounds contained non-conjugated rings or ring-systems and bromination. In contrast, the pH-sensitive compounds mainly featured phenolic and amine groups.

The presence of pH-sensitive compounds by phylogenetic group

Of the 23 compounds isolated from *Rhodophyta*, only one compound (*p*-hydroxyphenylethanol from *Gracilaria lemaneiformis*) was found to be affected by changes in pH within the defined range with a medium sensitivity (≤ 10% change) (Fig. 3). In contrast, three of the ten compounds isolated from *Chlorophyta* were found to be sensitive, including the two compounds ranked in the highest category "severely affected" (dopamine from *Ulva obscura* and 2-amino-3-hydroxy-3-sulfanylpropanoic acid from *Ulva pertusa*) and one compound in the "medium" category (dihydromenisdaurilide from *Ulva pertusa*). For *Phaeophyceae* 59% of the compounds were sensitive, with most of them ranking in the "mild" to "medium" categories, and two compounds (cystophloroketal B & D) were significantly affected with changes in protonation state by ≥ 30%.

Ecological functions affected by pH-sensitive compounds

Our modelling suggests that all functions could be at least partly affected by changes in pH, except for quorum-sensing disruption (QSD), which was represented by five pH-stable compounds belonging to the same class of halogenated furanones in *Delisea pulchra* (Fig. 4).

The antibiofilm (ABF) function was mostly conveyed by pH-stable compounds, only one of the compounds, *L*-proline in *Fucus vesiculosus*, was mildly pH sensitive. However, 40% of antimacrofouling (AMF) compounds were mildly to medium sensitive due to phenolic groups and all those sensitive compounds were found in brown algae, except for dopamine. Dopamine, the only AMF identified in green algae between 2010-2018, which is severely pH-sensitive due to the combined presence of a phenolic and an amino group, changes protonation state abundance by more than 50%.

The antimicroalgal (AA) compounds based on fatty acid and steroid moieties were pH-stable (13 of 25 compounds with this function), but all AA compounds in brown algae were medium to significantly sensitive due to phenolic groups. In red algae the only sensitive compound (*p*-Hydroxyphenylethanol) was affected with ≤ 10% change. In

contrast, the sensitive compounds in green algae based on amino acid derivatives and neurotransmitters were medium (20% change) to severely (56% and 75% change) affected by shifts in pH between 7.6 and 9.2.

50% of the compounds with antibacterial function (AB) were pH-stable and the other half mildly to significantly affected by pH changes. Sensitive compounds were exclusively found in brown algae and included proline in *Fucus* and phenolic compounds from *Sargassum* and *Cystoseira.*

For antifungal compounds (AF) two out of three (67%) were found to be pH sensitive and those two compounds from *Cystoseira* were significantly affected by more than 30% change in their protonation state abundance. However, the third compound with the same function, galactoglycerolipids, which stands for a whole compound class, was found to be pH insensitive and is also present in a brown alga, namely in *Sargassum muticum*.

Overall, only quorum-sensing disruption and antibiofilm activity can be seen as relatively pH-unaffected functions, while the other functions are all impacted by pH with a clear proportion of their conveying molecules possessing pH-sensitive functional groups.

Change in protonation state abundances and the predicted associated functionality

For most pH-sensitive compounds, the same protonation state can be observed to dominate across the pH range and increase with reducing pH (see for example Fig. 5 (b)). It can be assumed that the dominating protonation state likely is the bioactive form, which conveys the ecological function. This means that based on our results, the active forms of the molecules will be dominating across the pH range and even become more abundant in reduced pH. An exception is (-)- dihydromenisdaurilide, for which the dominating state decreases (Fig. 5 (a)) with lower pH, indicating a potential reduction in the effective functioning of this compound. For dopamine and 2-amino-3-hydroxy-3-sulfanylpropanoic acid (Fig. 5 (c) & (d)), the dominating protonation state at the upper level of the pH range is not the same as the state dominating at the lower pH level. This switch in dominating protonation state suggests a potentially significant impact on the associated function as the two states differ in their properties. Protonating a functional group adds a positive charge to the molecule, impacting the compound's net charge as well as its charge distribution. This can significantly impact their bioactivity or, in other words, their performance of the associated function, because charge distribution is an important factor in the membrane transport characteristics of a molecule (charged molecules cannot directly diffuse through the membrane) (Yang & Hinner 2015) and can further impact the compound's interaction with other molecules, such as receptor proteins or messengers of the receiving organism (Sheinerman et al. 2000; Hardege et al. 2011; Wyatt 2014).

Sensitivity of compounds to a decreased future pH range due to ocean acidification

By comparing the percentage of change in protonation state abundance for the current and future pH range, the potential impact of climate change on the macroalgal compounds and their properties can be assessed. All compounds previously stated to be pH-stable across the current pH range of 7.6 to 9.2 around macroalgae are found to be also unaffected by future pH conditions between pH 7.2 and 8.8. Of the 14 compounds identified as currently pH-sensitive, 12 are less affected in the future by changes in protonation state within the future pH range. All twelve compounds showed approximately half of the change in protonation in future conditions compared to the current pH range (see for example Fig. 5 (b) and (c)). 2-Amino-3-hydroxy-3-sulfanylpropanoic acid was found to be subject to the same severe amount of change in protonation in the current and future pH ranges Fig. 5 (d)). Only (-)-dihydromenisdaurilide (Fig. 5 (a)) will change significantly more within the future pH range, approximately double the amount compared to the change of 20% in today's pH range. Hence in 98% of compounds investigated here, future ocean conditions do not increase the protonation effects observed within the current natural pH range. In all sensitive compounds, the respective protonated form clearly dominated in the future pH range.

DISCUSSION

Macroalgae can cause a large variation of pH across a short spatial and temporal scale due to their photosynthetic activity and physiology (Wahl et al. 2016). As many of the ecological interactions including chemical defence of macroalgae are chemically mediated, one may expect that the compounds they use are likely to be pH-stable. We found that within a pH range of 7.6 to 9.2 around macroalgal thalli, 72% of the 50 investigated defence seaweed compounds are stable. However, 28% of the compounds, especially those with phenol and amino groups, were sensitive to deprotonation or protonation at a mild to a severe extent across the pH range near macroalgal thalli, which may potentially significantly impact their ecological functioning (Fig. 6) based on our calculations.

Implications of pH-sensitivity at a molecular functional level

Protonation or deprotonation of a compound at a particular group causes a shift in charge distribution and can potentially affect its conformation (Roggatz et al. 2016, 2019). Both of these properties play an important role in chemical interactions such as ligand-receptor interactions or membrane transport (Sheinerman et al. 2000; Yang & Hinner 2015) and a change in charge and/or conformation can consequently render a molecule biologically active or inactive. It has to be noted, that only a given proportion of the molecules present in the solution is changed with pH, as can be seen from the protonation state abundance curves (Fig. 5). However, for molecules with large changes, and in particular where the dominating form is changing across the pH range, significant implications for the functioning of the molecules can be assumed as the functionality of compounds is related to bioactivity thresholds.

Most pH-sensitive compounds increased in their neutral or zwitterionic states with decreasing pH. Functions that involve membrane-transport, e.g. antibacterial activity, may be limited to neutral protonation states as a net charge may prevent transport across the hydrophobic membrane. For cystophloroketals, for example (Fig. 5(b)), the fully protonated, neutral state dominates across the pH range and can be assumed to be the active form. Based on our calculations, the higher abundance of this form at the lower end of the relevant pH range means that the compounds' antibacterial, antifungal, and antimicroalgal functions are likely to improve with decreasing pH. If, however, the deprotonated state at one of the phenolic groups were to be the active form, the compounds' activity would significantly decrease with decreasing pH.

For only two of the 50 compounds (dopamine from *Ulva obscura* and 2-amino-3-hydroxy-3-sulfanylpropanoic acid from *Ulva pertusa*) a switch of the dominating protonation state could be observed within the defined pH range. Dopamine is produced by *Ulva obscura*, a bloom-forming green alga that occurs from the mid intertidal to the shallow subtidal zones on North Pacific and North Atlantic shores. Its concentrations in the alga are approximately 0.5–1% of the alga's fresh biomass and can exceed 500 μM in seawater when it is released by damaged algae tissue after desiccation. Dopamine was found to inhibit *Fucus* germination at concentrations above 5 μM, *Ulva* growth at concentrations above 50 μM, and affect larval development of several invertebrates (Rivera Vázquez et al. 2017; Van Alstyne et al. 2014). Large-scale release of dopamine by *U. obscura* following stressful environmental conditions could significantly affect co-occurring species in intertidal pools as well as intertidal and shallow subtidal marine communities where the alga can form large blooms (Van Alstyne et al. 2014). Dopamine has also been found to function as an anti-grazing compound in *Ulva obscura*, which may contribute to the formation and persistence of harmful *Ulva* blooms in north-eastern Pacific coastal waters (Van Alstyne et al. 2014). From our calculations we predict that a change in the dopamine protonation state by more than 50%, as revealed in this study, could significantly alter its functioning. We predict that a change in pH may therefore impact the defence of *Ulva* against other macroalgal competitors and grazers in space-limited benthic environments with resulting community re-

structuring. However, the mechanistic link between a change in protonation state and dopamine's functionality as well as the associated ecological effects remain to be investigated experimentally.

In general, for pH-sensitive compounds, a change in pH close to macroalgae thalli caused by the photosynthetic activity coupled to the light/dark cycle can potentially act as an activity switch. By increasing or decreasing the relative abundance of the active compound form, the respective function of a compound can be up- or down-regulated. Hence, substances with an active form mainly present in high pH during light could be rendered less functional by lower pH through respiration at night and *vice versa*. It remains to be clarified which of the individual protonation states represents the biologically active form of each compound, as this may be, but not necessarily has to be, the dominating form. For compounds with multiple functions, the active states may also differ between the different functions, meaning the neutral form may convey antibacterial function while the deprotonated form may possess the antimicroalgal activity. Here we have identified those compounds potentially sensitive to pH. However, determining the biologically active forms using bioassays in different pH conditions for the individual compounds deserves future laboratory investigations.

It has to be noted that large fluctuations in temperature may affect the compounds' sensitivity to pH as the dissociation constants for amine and phenolic groups are reduced with increasing temperature (Reijenga et al. 2007). For the pH range and compounds investigated here, however, the obtained sensitivity scores would only change for temperature increases of more than 10°C. With increasing temperature protonated states of most pH-sensitive compounds become more abundant, while decreasing temperatures reduce observed differences for the investigated pH range. Reduction by 10°C affects protonation state abundance by less than 10% but would drop dopamine, chromanols A-F and Cystophloroketals B & D just below the thresholds of their current categories to a less sensitive one. Our study further employs calculated pK_a constants, which come with an associated error margin compared to experimentally determined values in relevant conditions. The error margin of calculated constants does not significantly alter our results in terms of classifying the compounds as pH-sensitive or pH-stable. However, it presents a significant limitation of this current study and experimental determination of constants for relevant compounds within natural temperature fluctuations should be the first step of any future investigation to validate our predictions.

Not all ecological functions are equally sensitive to pH fluctuations

As described earlier the ecological functions associated in the literature with the compounds used in our dataset to calculate molecular protonation changes include antibacterial, antifungal, antimicroalgal, antibiofilm, antimacrofouling, and quorum sensing disruption, but not all were found to be equally stable to fluctuations in pH.

Quorum sensing disruption was found to be the only function that is conveyed solely by pH-stable compounds, indicating that under current and future pH fluctuations macroalgae like the red alga *Delisea pulchra* capable of producing such compounds may be able to effectively disrupt such cell to cell bacterial communication avoiding intense bacterial colonization. The same holds true for most of the compounds with antibacterial function, except for *L*-proline in *Fucus vesiculosus*, which was mildly pH-sensitive. The pH stability of these essential ecological functions matches our expectation. Overgrowth by bacterial biofilms can be of significant cost to the macroalgae, e.g. by reducing its photosynthetic activity (Wahl et al. 2012) and a pH-dependency of these essential ecological functions may have negative consequences for the algae.

However, all other chemically-mediated defence functions are conveyed by 40 to 67% pH-sensitive compounds, but we found no function to be mediated solely by pH-sensitive compounds. From an ecological point of view, functional redundancy is only achieved if the same macroalgal species contains pH-stable compounds with the same function. This can be seen, for example, in the chlorophyte *Ulva pertusa*, where the antimicroalgal function of the

371 pH-sensitive compounds 2-Amino-3-hydroxy-3-sulfanylpropanoic acid and (-)-dihydromenisdaurilide is also
372 conveyed by a bouquet of seven other compounds that are unaffected by pH changes. The same can be observed for
373 the brown algae *Fucus vesiculosus*, where the antibacterial function conveyed by the pH-sensitive *L*-proline is
374 redundantly covered by pH-insensitive DMSP and fucoxanthin.
375
376 In theory the combination of pH-sensitive and pH-insensitive compounds in a bouquet should ensure continuous
377 protection against bacteria and biofilm colonisation in all pH conditions. In other brown algae included in our study,
378 however, all identified compounds were found to be pH-sensitive. For *Cystoseira tamariscifolia* and *Sargassum*
379 *horneri* the antibacterial, antifungal, antimicroalgal, and antimacrofouling functions are not redundant and all
380 compounds are medium to significantly affected by pH. At maximum or minimum pH during a diurnal cycle, these
381 brown algae may therefore have altered antifouling capacity. This also suggests that such antifoulings may fluctuate
382 at a sub-circadian scale and not just at a seasonal scale as known for other seaweeds (Saha and Wahl 2013).
383 Macroalgal holobionts are complex communities and there are many more chemically-mediated interactions taking
384 place in these. The current study is limited to signalling compounds that have a defensive function. Profouling
385 compounds that macroalgae use to gain an advantage as well as bioavailability of essential trace metals may well be
386 pH dependent (Millero et al. 2015), and present examples for future modelling opportunities once experimental data
387 exist to support our approach.
388
389 *High functional redundancy at phylum level*
390 The pH-sensitivity at phylum level can give insights into impacts at a wider ecosystem-relevant level. The
391 interspecific ecological interactions of *Rhodophyta* are barely affected by changing pH. Only *p*-
392 hydroxyphenylethanol produced by the agarophyte *Gracilaria lemaneiformis* will be medium affected, but its
393 antimicroalgal function is conveyed redundantly by other compounds in the bouquet (see Table 3, Fig. 6), making
394 the interactions of this red algae with other organisms pH-insensitive. In *Chlorophyta*, 30% of the investigated
395 compounds were pH-sensitive whilst amongst the compounds isolated from *Phaeophyceae*, 59% are pH-sensitive
396 and susceptible to de-/protonation. Many of these sensitive compounds also possess multiple biological functions,
397 making this phylum the most affected one. All antimicroalgal compounds, most of the macrofouling-preventive
398 compounds, and half of the antibacterial compounds are pH-sensitive. It has to be noted that the results obtained in
399 this study are limited only to a selected number of source species, a small amount of fully chemically identified
400 individual compounds with known associated functions for studies conducted between 2010-2018. This can cause
401 an unintended selection bias. Studies on bioactive compounds are often limited to species with high abundance and
402 wide biogeographical distribution to allow for the production of sufficient amounts of extract for bioassay-guided
403 fractionation and compound identification. Hence, abundant foundation macroalgae, such as *Fucus vesiculosus* in
404 the NE Atlantic, are prioritised and best studied. In addition, most of the studies focus on reporting a specific group
405 of compounds with a specific property or function (e.g. halogenated furanones or micro-algal compounds against
406 red tides).
407 While our calculation can provide a first indication for bioactive compounds, the excretions from macroalgae
408 present highly diverse mixtures. It is not possible to retrieve the exact chemical structure and properties of one
409 specific bioactive compound within such mixtures conveying a given function without employing additional, often
410 lengthy, and complicated, extraction steps. We, therefore, limited the selection of bioactive entities in our list to
411 actual chemically identified individual compounds and the functionality to defence compounds. While this makes a
412 more meaningful comparison possible, it may also result in some compound classes being represented by multiple

compounds (e.g. halogenated furanones, chromanols, etc.) and therefore appear to be of higher influence. Our results here should be interpreted with the necessary caution.

Phaeophyceae compounds are particularly pH-sensitive

Almost 60% of the brown algae compounds in our list (see Table 3, Fig. 6) are sensitive to pH changes and for two of the five species only pH-sensitive substances are known, suggesting a particular sensitivity of brown algae to changes in pH. Phlorotannins are not included in our dataset because their complex microspeciation and keto-enol-tautomerisation make simple quantitative scoring of their pH-sensitivity as performed in this study impossible. However, the monomer phloroglucinol, on which all phlorotannin structures are based on, contains multiple phenol groups. This indicates a high potential for these compounds to be pH-sensitive, analogue to the cystophloroketals or dopamine, for example. With their antimicroalgal, antibacterial, antimacrofouling, and antigrazing functions, phlorotannins are essential in defending *Phaeophyceae* against over-colonisation. Given their verified presence in a large number of different brown algae, including the *Cystoseira*, *Sargassum,* and *Fucus* genera in our data set, an important new question arises: why would brown algae employ a pH-sensitive defence system when the algae cause large pH fluctuations themselves? Possible lines of inquest could investigate whether (a) there is no functional difference for protonation states of phenolic compounds, (b) phenolic compounds are a metabolically cheap but pH-sensitive, hence less effective, defence used by algae, or (c) the sensitivity of the compounds is used to switch active defence on/off in conjunction with a respective pH condition (e.g. high pH during the day). Recent work by Gaubert et al. (2020) on the brown alga *Lobophora rosacea* demonstrated that long-term, as well as short-term exposure to low pH conditions, causes significant quantitative changes to the compounds comprising the algae's metabolome. Lobophorenols, which are known to induce allelopathic activity against corals, were found to be significantly decreased in concentration in lower pH conditions. The authors attributed this to a potential shift of the metabolism necessary to maintain essential processes (growth, reproduction, and homeostasis) in lower pH or by an increased rate of release of these compounds into the surroundings (Gaubert et al. 2020). Quantitative changes and a difference in release rates combined with a pH-associated reduction or increase of the respective bioactive form as presented in our study may profoundly affect the dynamics of the interactions mediated by these compounds.

Future ocean conditions favour protonated forms and affect how much compounds change with pH

Ocean acidification is predicted to cause a shift of the open ocean average pH range by -0.4 pH units within this century assuming a business as usual scenario, which we found to increase the abundance of protonated states of the defence compounds. A future uniform shift would also reduce the relative extent of changes in protonation state abundances. In other words, within the range of today's naturally fluctuating pH, more of the pH sensitive compounds change in their protonation state abundance between more and less protonated forms than in the shifted future open ocean average pH range, where the protonated forms would dominate. However, this only holds true assuming a uniform shift of the pH range and does not take into account that pH fluctuation levels might also increase in severity and change in timing with potentially prolonged phases of lower pH in the future (Takeshita et al. 2015). Additionally, amplitudes of high and low pH are also known to increase especially near coast and in estuaries and throughout the seasons with significantly lower pH in winter nights as CO_2 solubility is temperature dependent (Landschützer et al. 2018).

As discussed above, we know very little about the identity of the bioactive form for most macroalgal compounds. If the bioactive form conveying the respective function is the protonated state, its abundance will increase in future ocean conditions. In contrast, if the compound needs to be deprotonated to convey its function, it will be significantly reduced in its abundance in future conditions. Reduced concentrations of a bioactive compound can

lead to a shortfall in the number of molecules released to reach the threshold concentration required to trigger a biological function. Future conditions could therefore alter a compound's functioning and this theory deserves future experimental investigation (Porteus et al. 2021). Such effects on bioactive compounds are highly compound specific and currently in a very early stage of experimental investigation.

Future oceans are also predicted to be warmer and the frequency and duration of heat waves is expected to increase (Fifth IPCC report, IPCC, 2021). It was found that high temperature conditions can lead to bleached thalli and lower levels of antibacterial defence in the red seaweed *Delisea pulchra* as its production of halogenated furanones is decreased (Campbell et al. 2011). Severe conditions of temperature and salinity can also lead to a decreased production of herbivory-inhibiting and settlement-preventing compounds in *Laurencia dendroidea* (Sudatti et al. 2011). However, extreme events like heatwaves were found to not impair the antifouling defence of the brown seaweed *Fucus vesiculosus* against bacteria (Saha et al. 2020). In addition to validating our prediction on the pH-sensitivity of the respective defensive compounds, it will be necessary to assess the impact of temperature in more detail, particularly on compound production, as this may add to or overwrite any pH-associated alterations.

CONCLUSION

Based on our calculations, the majority of macroalgal compounds were found to be insensitive to changes in pH near the surface of the algal thalli. The naturally large pH fluctuation at the small scale around the macroalgal boundary layer may have led to the development of this array of pH-insensitive compounds. The macroalgal photosynthetic activity results in a diurnal pH pattern dependent on the light/dark cycle, which can alter compound's activity through the pH-dependent abundance of the bioactive protonation state. This delicate interplay of pH-sensitive and stable interactions may be affected by future ocean conditions~~,~~. The abundance of a variety of functionally redundant pH-stable compounds in bouquets allows for interactive functions, such as antibacterial and antimacrofouling activity, to prevail for most algal species in our study. However, we also identified some brown algae species relying solely on pH-sensitive compounds for some antifouling functions. The extent of the compounds' pH-sensitivity in natural temperature conditions, and their actual functioning (bioactive forms) still need to be validated experimentally due to the modelling method-associated limitations of our study. As the warming of our oceans may also have a considerable impact on the sensitivity of organisms and their ability to produce defensive compounds, as well as affecting the pH-sensitivity of compounds, we would like to highlight that effects of warming and heatwaves could add to the effects of ocean acidification and should be investigated. More work is further needed to identify the bioactive protonation states of the signalling compounds, assess the functional implications of natural pH fluctuations *in situ,* and gain insight into why some macroalgae use pH-sensitive compounds that even alter compound functionality today during natural day/night cycles.

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Author contributions

CCR, JDH and MS planned and designed the research. CCR performed the research and analysed the data. Both, CCR and MS, collected and interpreted the data. CCR, JDH and MS wrote the manuscript. CCR and MS contributed equally.

Data availability

Any data is available from the corresponding or the first author on request and will be happily made available.

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Figures legends

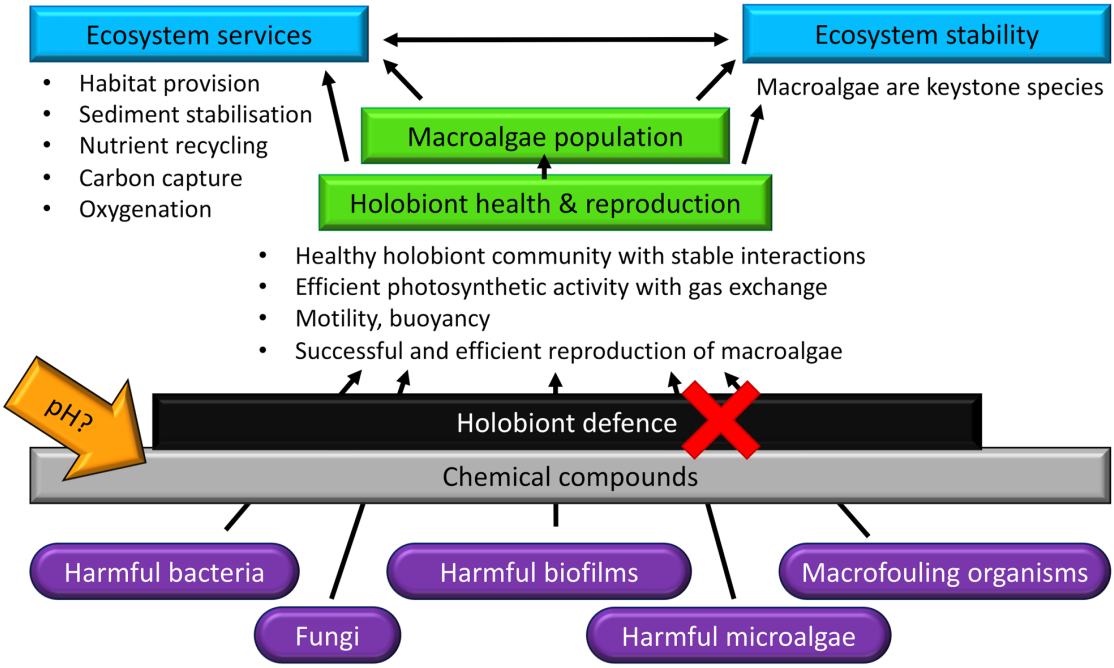


Figure 1: ***Schematic overview of ecosystem services provided by macroalgal-holobionts and how they defend themselves against harmful micro- and macro-organisms using chemical compounds.*** As this chemical line of defence is exposed to changing environmental conditions, we investigate if and how pH affects the compounds involved in these critical interactions.

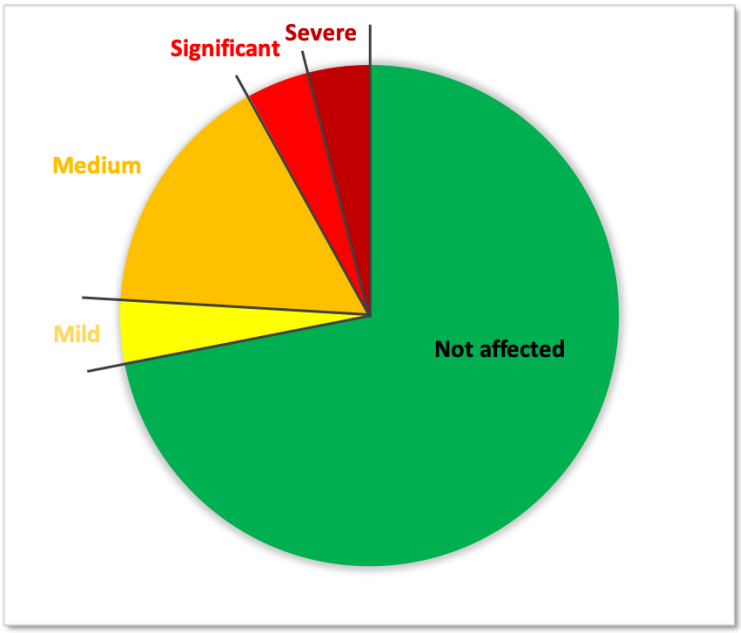


Figure 2: ***pH-sensitivity scores of all 50 investigated compounds*** *based on their change in protonation within the natural, relevant range of pH 7.6 to 9.2. Compounds are classed according to none, mild (≤ 5%), medium (≥ 5 to ≤ 30%), significant (≥ 30 to ≤ 50%), and severe (≥ 50%) change in protonation state across the pH range.*

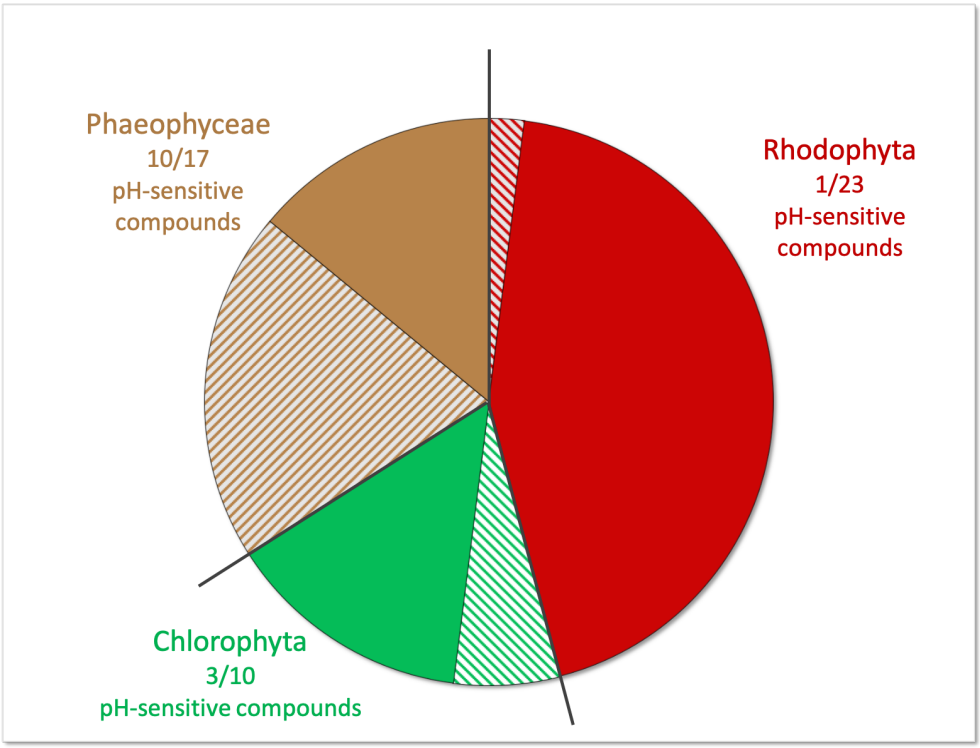


*Figure 3**: Sensitivity of compounds to pH change for the three investigated phylogenetic groups.** Compounds stable between pH 7.6 and pH 9.2 are indicated by solid colours while compounds sensitive to changes within this pH range are indicated by shading. Red represents the 23 compounds isolated for Rhodophyta, green the ten compounds found for Chlorophyta and brown the 17 compounds identified in Phaeophyceae.

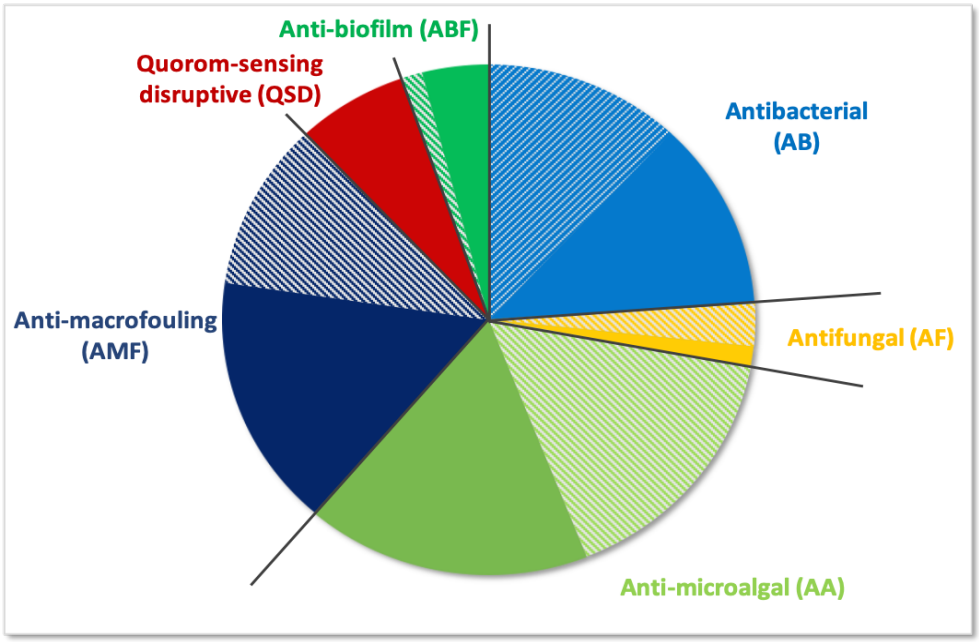


*Figure 4: **Sensitivity of compounds to pH change based on their different functions.** Compounds are sorted based on their functions (multiple functions per compound possible). Compounds stable between pH 7.6 and pH 9.2 are indicated by solid colours, compounds sensitive to changes within this pH range are indicated by the shading.*

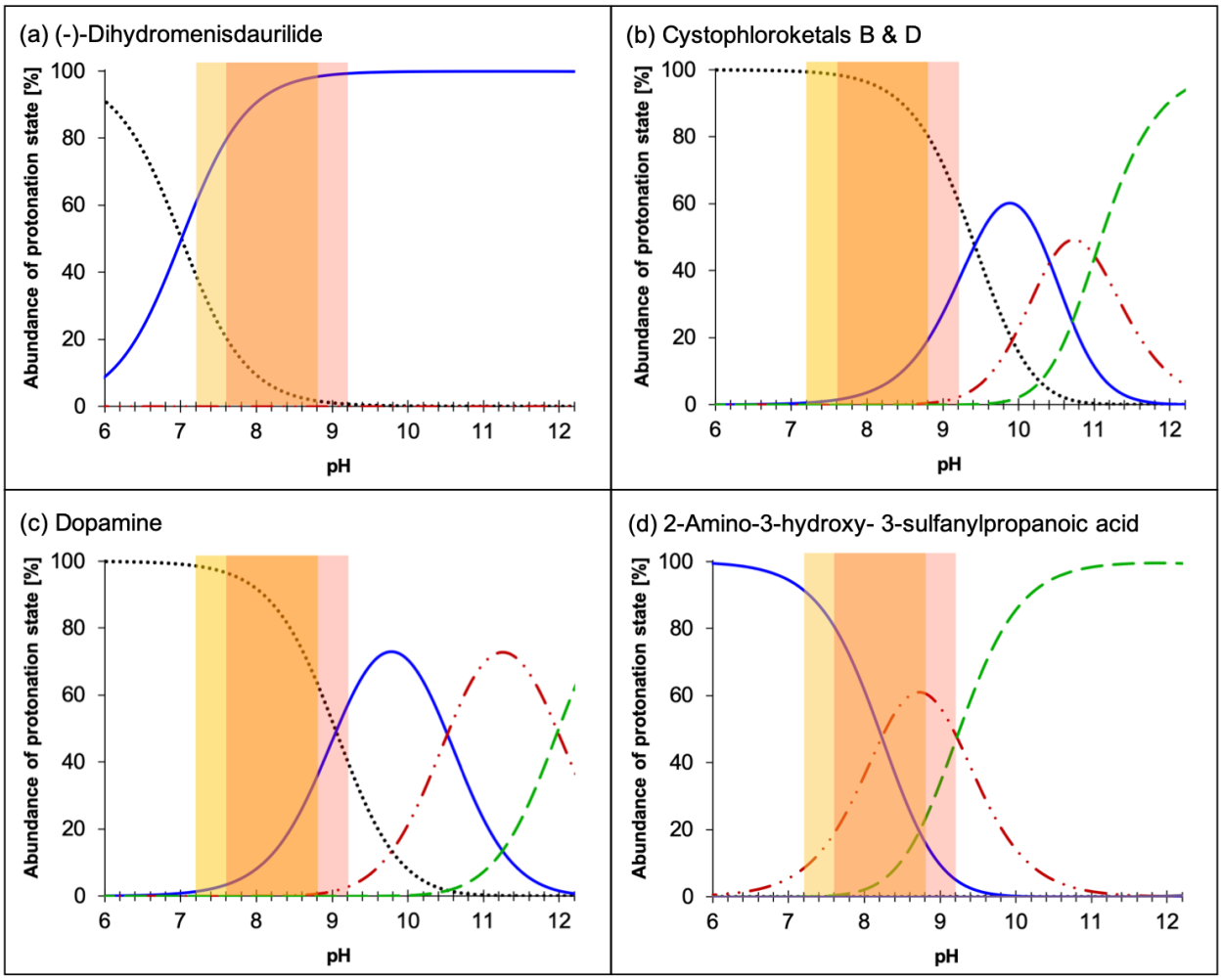


Figure 5: ***Examples of protonation state abundance curves between pH 6 and 12 for four compounds rated as pH-sensitive*** *between pH 7.6 and pH 9.2 and ranked according to the change in protonation state(s): (a) (-)-Dihydromenisdaurilide (20% change), (b) Cystophloroketals B & D (37% change), (c) Dopamine (56% change), (d) 2-Amino-3-hydroxy- 3-sulfanylpropanoic acid (75% change). Green-dashed (fully deprotonated), red-dashed-dotted (one protonated group), blue-solid (two protonated groups) and black-dotted (fully protonated) lines represent different protonation states with different overall charge, respectively. The current and future pH ranges are indicated by the red (pH 7.6 to 9.2) and yellow (pH 7.2 to 8.8) shaded areas.*

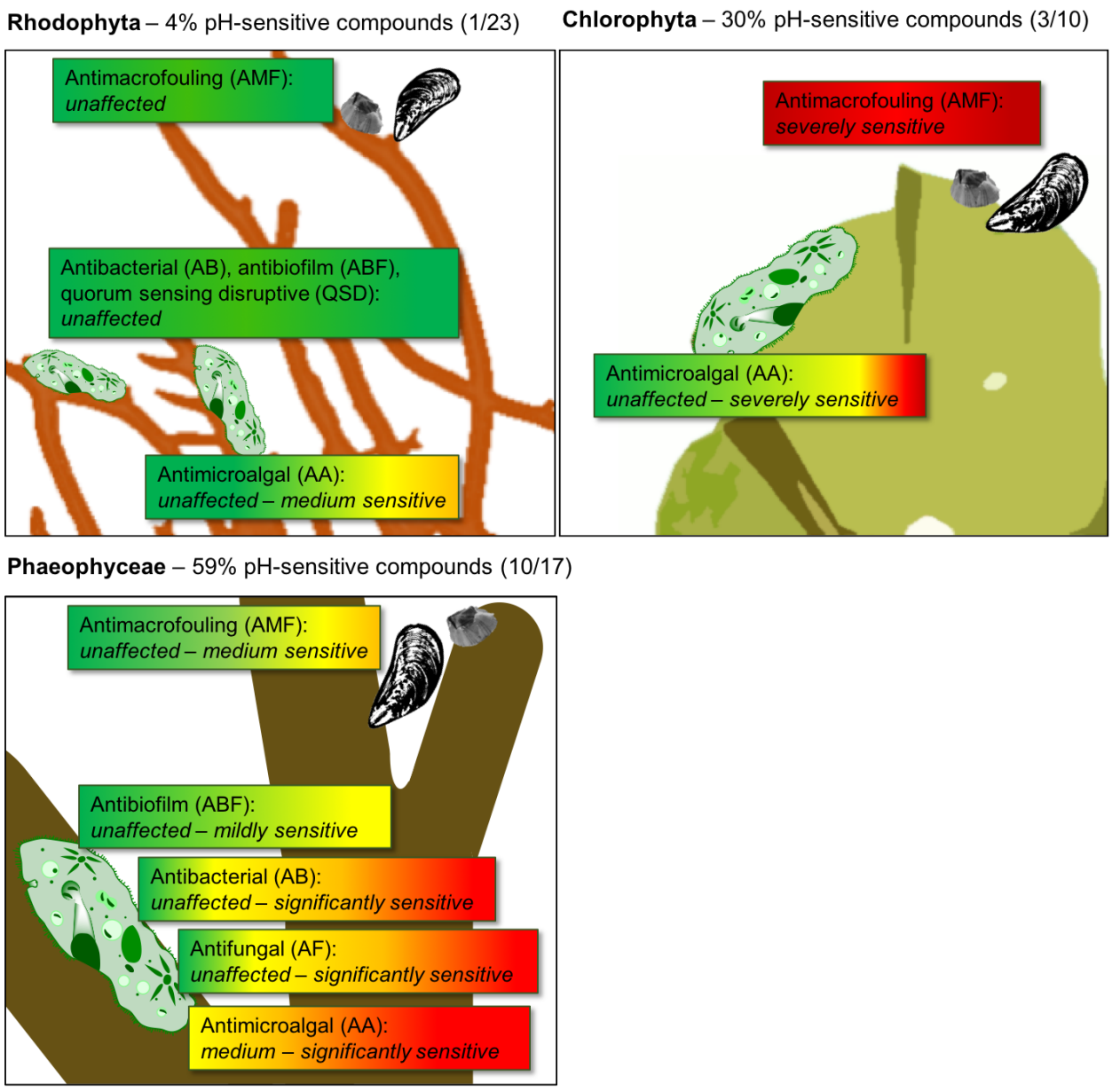
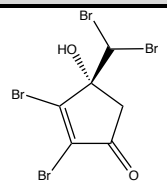
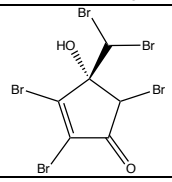
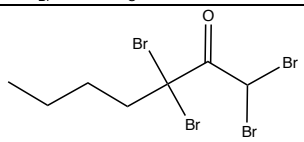
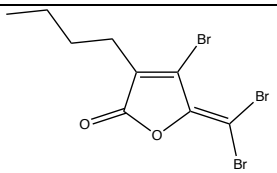
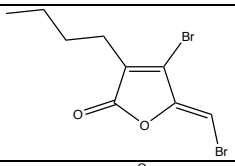
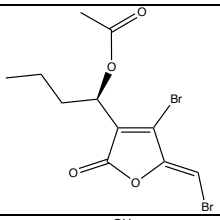
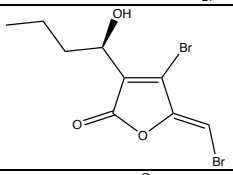
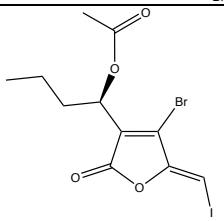
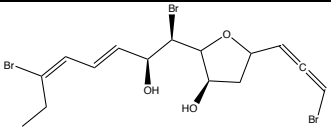


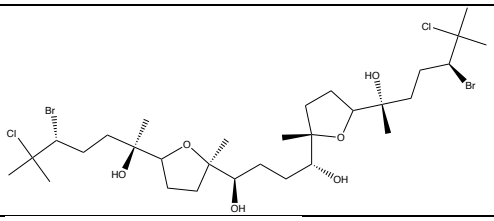
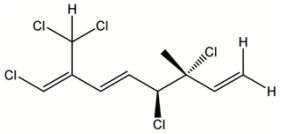
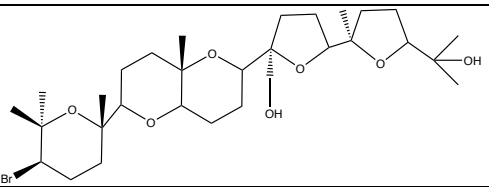
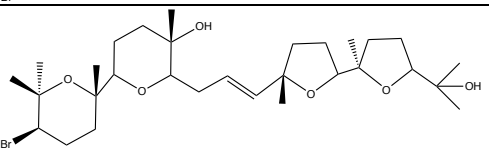
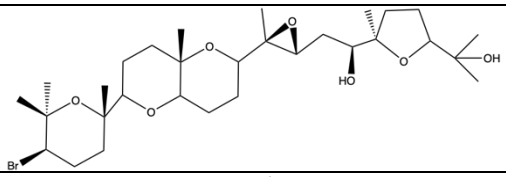
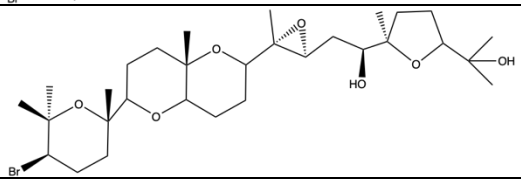
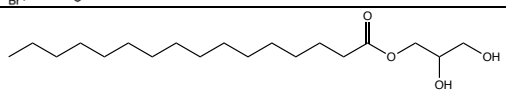
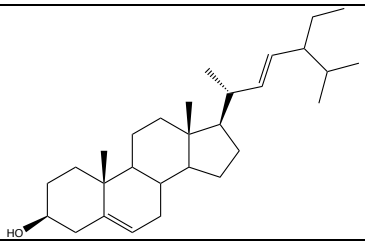
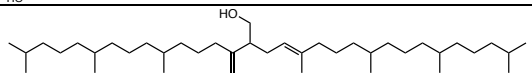
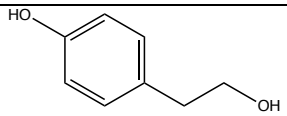
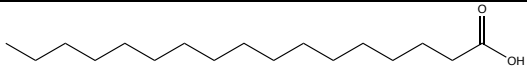
Figure 6: ***pH-sensitivity of ecological functions mediated by the investigated macroalgae compounds*** *for red, green and brown macroalgae. Macrofouling is represented by mussel and barnacle icons; microfouling, interactions with bacteria, fungi, microalgae and biofilms are represented by the biofilm icon. Labels of functions are colour coded according to their sensitivity in line with the scoring in Table 1: green – unaffected and insensitive to pH changes, yellow – mildly sensitive, orange – medium sensitive, red – significantly sensitive, and dark red – severely affected.*

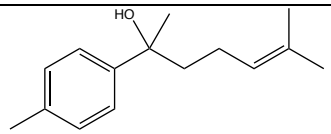
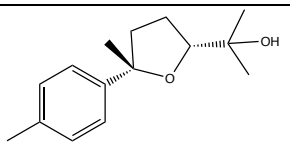
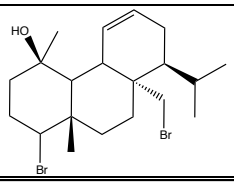
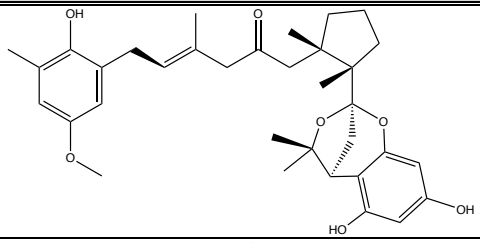
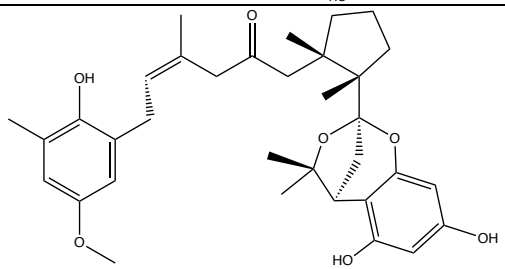
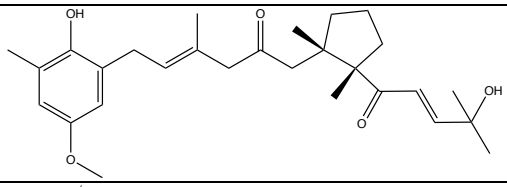
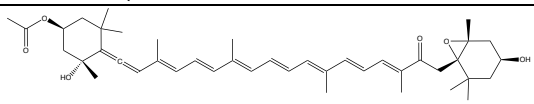
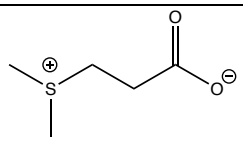
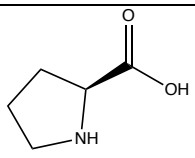
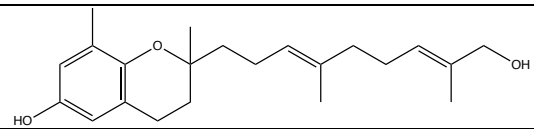
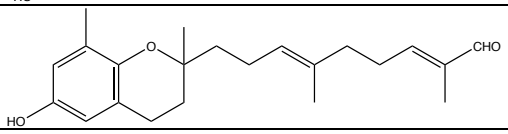
Table 1: pH-Sensitivity scoring of compounds based on change in the abundance of different protonation states within the defined range. Colour code used as indication of sensitivity in Table 3.

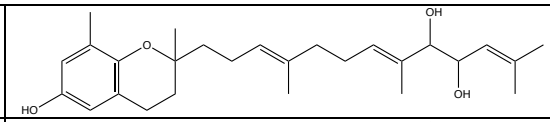
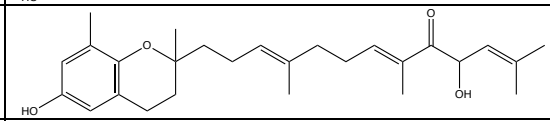
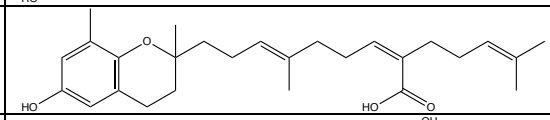
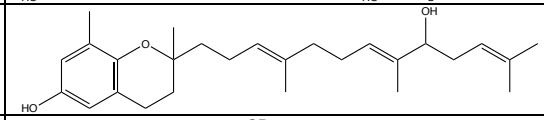
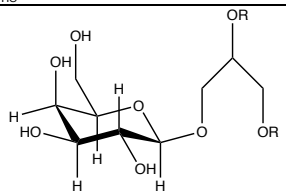
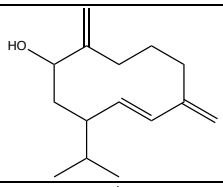
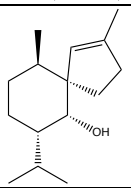
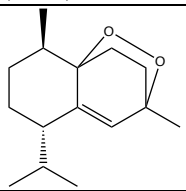
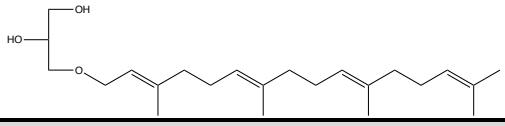
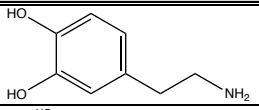
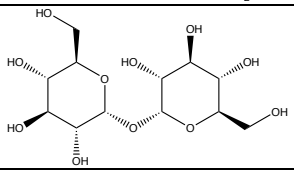
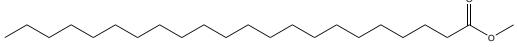
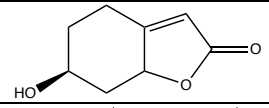
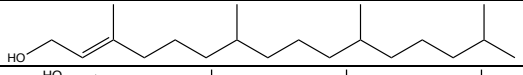
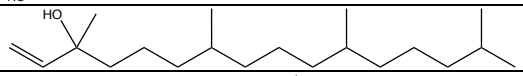
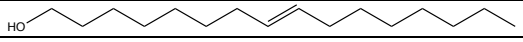
Change in protonation state abundance	Sensitivity score
none	insensitive
≤ 5%	mildly affected
≥ 5 to ≤ 30%	medium affected
≥ 30 to ≤ 50%	significantly affected
≥ 50%	severely affected

Table 2. Macroalgal compounds mediating interactions between macroalgae and other organisms

Compound No.	Source/ Species	Biogenic compound(s)	Bioactivity	Structure(s)	Reference
Rhodophyta					
1	*Asparagopsis taxiformis*	Mahorone	AB		Greff *et al.*, 2014
2	*Asparagopsis taxiformis*	5-Bromomahorone	AB		Greff *et al.*, 2014
3	*Bonnemaisonia hamifera*	1,1,3,3-Tetrabromo-2-heptanone	ABF		Persson *et al.*, 2011
4	*Delisea pulchra*	Halogenated furanone$_1$	QSD		Harder *et al.*, 2012
5	*Delisea pulchra*	Halogenated furanone$_2$	QSD		Harder *et al.*, 2012
6	*Delisea pulchra*	Halogenated furanone$_3$	QSD		Harder *et al.*, 2012
7	*Delisea pulchra*	Halogenated furanone$_4$	QSD		Harder *et al.*, 2012
8	*Delisea pulchra*	Halogenated furanone$_5$	QSD		Harder *et al.*, 2012
9	*Laurencia* sp.	Omaezallene	AMF		Umezawa *et al.*, 2014

10	*Laurencia* sp.	Intricatetraol	AMF		Umezawa *et al.*, 2014
11	*Laurencia translucida*	Pentachlorinated monoterpene	AMF		Paradas *et al.*, 2016
12	*Laurencia viridis*	28-Hydroxysaiyacenol B	AMF		Cen-Pacheco *et al.*, 2015
13	*Laurencia viridis*	Saiyacenol C	AMF		Cen-Pacheco *et al.*, 2015
14	*Laurencia viridis*	15,16-Epoxythyrsiferol A	AMF		Cen-Pacheco *et al.*, 2015
15	*Laurencia viridis*	15,16-Epoxythyrsiferol B	AMF		Cen-Pacheco *et al.*, 2015
16	*Gracilaria lemaneiformis*	Glycerol monopalmitate	AA		Sun *et al.*, 2017
17	*Gracilaria lemaneiformis*	Stigmasterol	AA		Sun *et al.*, 2017
18	*Gracilaria lemaneiformis*	15-Hydroxymethyl-2, 6, 10,18, 22, 26, 30-heptamethyl-14-methylene-17-hentriacontene	AA		Sun *et al.*, 2017
19	*Gracilaria lemaneiformis*	*p*-Hydroxyphenyl-ethanol	AA		Sun *et al.*, 2017
20	*Gracilaria lemaneiformis*	Margaric acid	AA		Sun *et al.*, 2017

21	*Gracilaria lemaneiformis & Porphyra yezoensis*	Gossonorol	AA		Sun *et al.*, 2017, 2018a
22	*Gracilaria lemaneiformis & Porphyra yezoensis*	7,10-Epoxy-ar-bisabol-11-ol	AA		Sun *et al.*, 2017, 2018a
23	*Sphaerococcus coronopifolius*	Bromosphaerol	AMF		Piazza *et al.*, 2011
Phaeophyceae					
24	*Cystoseira tamariscifolia*	Cystophloroketal B	AA, AB, AF		El Hattab *et al.*, 2015
25	*Cystoseira tamariscifolia*	Cystophloroketal D	AA, AB, AF		El Hattab *et al.*, 2015
26	*Cystoseira tamariscifolia*	Monocyclic meroditerpenoid	AMF		El Hattab *et al.*, 2015
27	*Fucus vesiculosus*	Fucoxanthin	AB, ABF		Wahl *et al.*, 2010; Saha *et al.*, 2011; Lachnit *et al.*, 2013
28	*Fucus vesiculosus*	Dimethyl-sulfoniopropionate (DMSP)	AB, ABF		Wahl *et al.*, 2010; Saha *et al.*, 2012; Lachnit *et al.*, 2013
29	*Fucus vesiculosus*	Proline	AB, ABF		Wahl *et al.*, 2010; Saha *et al.*, 2012; Lachnit *et al.*, 2013
30	*Sargassum horneri*	Chromanol A	AA, AB, AMF		Cho, 2013
31	*Sargassum horneri*	Chromanol B	AA, AB, AMF		Cho, 2013

32	*Sargassum horneri*	Chromanol C	AA, AB, AMF		Cho, 2013
33	*Sargassum horneri*	Chromanol D	AA, AB, AMF		Cho, 2013
34	*Sargassum horneri*	Chromanol E	AA, AB, AMF		Cho, 2013
35	*Sargassum horneri*	Chromanol F	AA, AB, AMF		Cho, 2013
36	*Sargassum muticum*	Galactoglycero-lipids (compound class)	AB, AF, AMF		Plouguerné *et al.*, 2010
37	*Taonia atomaria*	Sesquiterpene 1	AB, AMF		Othmani *et al.*, 2016
38	*Taonia atomaria*	Sesquiterpene 2	AB, AMF		Othmani *et al.*, 2016
39	*Taonia atomaria*	Sesquiterpene 6	AB, AMF		Othmani *et al.*, 2016
40	*Taonia atomaria*	sn-3-O-(geranyl-geranyl)glycerol	AB		Othmani *et al.*, 2016
Chlorophyta					
41	*Ulva obscura*	Dopamine	AA, AMF		Van Alstyne *et al.*, 2014
42	*Ulva pertusa*	Trehalose	AA		Sun *et al.*, 2018b
43	*Ulva pertusa*	Methyl behenate	AA		Sun *et al.*, 2018b
44	*Ulva pertusa*	(-)-Dihydromenis-daurilide	AA		Sun *et al.*, 2018b
45	*Ulva pertusa*	Phytol	AA		Sun *et al.*, 2018b
46	*Ulva pertusa*	Isophytol	AA		Sun *et al.*, 2018b
47	*Ulva pertusa*	8-Hexadecenol	AA		Sun *et al.*,

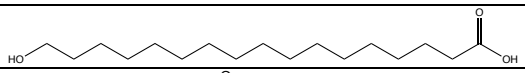
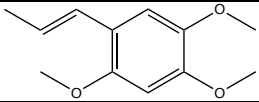
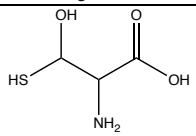
					2018b
48	*Ulva pertusa*	17-Hydroxyhepta-decanoic acid	AA		Sun *et al.*, 2018b
49	*Ulva pertusa*	Trans-asarone	AA		Sun *et al.*, 2018b
50	*Ulva pertusa*	2-Amino-3-hydroxy-3-sulfanylpropanoic acid	AA		Sun *et al.*, 2018b

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Table 3: Sensitivity scores of macroalgal compounds to pH change across the natural range between pH 7.6 and 9.2. Sensitive compounds are classed and colour-coded according to Table 1 and highlighted in bold. Bioactive functions are given in brackets.

Rhodophyta			
Asparagopsis taxiformis	Mahorone (AB)	5-Bromomahorone (AB)	
Bonnemaisonia hamifera	1,1,3,3-Tetrabromo-2-heptanone (ABF)		
Delisea pulchra	Halogenated furanone$_{1\text{-}5}$ (QSD)		
Laurencia sp.	Omaezallene (AMF)	Intricatetraol (AMF)	
Laurencia translucida	Pentachlorinated monoterpene (AMF)		
Laurencia viridis	28-Hydroxysaiyacenol B (AMF)	Saiyacenol C (AMF)	15,16-Epoxythyrsiferol A (AMF)
	15,16-Epoxythyrsiferol B (AMF)		
Gracilaria lemaneiformis	Glycerol monopalmitate (AA)	Stigmasterol (AA)	Margaric acid (AA)
	p*-Hydroxyphenylethanol (AA)** ***Medium sensitivity *(<10% change)*	15-Hydroxymethyl-2, 6, 10,18, 22, 26, 30- heptamethyl-14-methylene-17-hentriacontene (AA)	
Gracilaria lemaneiformis & Porphyra yezoensis	Gossonorol (AA)	7,10-Epoxy-ar-bisabol-11-ol (AA)	
Sphaerococcus coronopifolius	Bromosphaerol (AMF)		
Phaeophyceae			
Cystoseira tamariscifolia	**Cystophloroketal B (AA, AB, AF)** ***Significant sensitivity*** *(>30% change)*	**Cystophloroketal D (AA, AB, AF)** ***Significant sensitivity*** *(>30% change)*	**Monocyclic meroditerpenoid (AMF)** ***Mild sensitivity*** *(<5% change)*
Fucus vesiculosus	Fucoxanthin (AB, ABF)	Dimethylsulfoniopropionate (DMSP) (AB, ABF)	**Proline (AB, ABF)** ***Mild sensitivity*** *(<5% change)*
Sargassum horneri	**Chromanol A-F (AA, AB, AMF)** ***Medium sensitivity*** *(10% change)*		
Sargassum muticum	Galactoglycerolipids (AB, AF, AMF)		
Taonia atomaria	Sesquiterpene$_1$ (AB, AMF)	Sesquiterpene$_2$ (AB, AMF)	Sesquiterpene$_6$ (AB, AMF)
	sn-3- O-(geranylgeranyl)glycerol (AB)		
Chlorophyta			
Ulva obscura	**Dopamine (AA, AMF)** ***Severe sensitivity*** *(>50% change)*		
Ulva pertusa	Trehalose (AA)	Methyl behenate (AA)	Isophytol (AA)
	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (AA)	**(-)-Dihydromenisdaurilide (AA)** ***Medium sensitivity*** *(20% change)*	**2-Amino-3-hydroxy-3-sulfanylpropanoic acid (AA)** ***Severe sensitivity*** *(75% change)*
	17-Hydroxyheptadecanoic acid (AA)	Trans-asarone (AA)	8-Hexadecenol (AA)

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