ALLELOPATHY IN MACROALGAE: ECOLOGICAL PRINCIPLES, RESEARCH OPPORTUNITIES AND PITFALLS REVIEWED

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Abstract

Macroalgae are a vast source of bioactive metabolites, some of which are produced as a defence against competing and/or fouling species, i.e., allelochemicals. As both the occurrence of allelopathy in seaweed, and its underlying mechanisms, are understudied, we summarised the current knowledge on this phenomenon, as well as to elucidate opportunities and challenges in this emerging field. We mark out which biotic and environmental factors govern the stability and durability of allelopathic interactions, and which of them might confound conclusions on the absence/presence of allelopathy in macroalgae. We compiled information of the taxonomic position of 138 putative allelopathic species of brown, green, and red seaweed, as well as the identity of the compounds they produce. Additionally, we characterise which physiological processed are likely targeted by aquatic allelochemicals. In summary, this work updates our understanding of both the occurrence of allelopathy in seaweed on a global scale as their

allelochemicals affecting competitors, bringing forth recommendations for future research on this topic.

Keywords: allelopathy; allelochemicals; chemical defence; biodiscovery marine ecology; species interactions; marine ecology; antifouling

1. Introduction

In the euphotic zone, competition among sessile photoautotrophic organisms for space, light and nutrients is particularly fierce and acute (Chadwick and Morrow, 2011; Dayton, 1971). Competition has been identified as a pivotal process shaping the structure and functioning of communities and ecosystems, as well has impacted the ecology and evolution of species (Chadwick and Morrow, 2011; Dayton, 1971; Sousa, 1979). In coral reefs for example, coral-macroalgal competition can be a significant factor of the biodiversity, structure, and functioning of the benthic community (e.g., McCook et al., 2001; Miller, 1998). Moreover, while competing for space, seaweeds themselves become a substrate for colonisation by other organisms, ranging from prokaryotes to animals, whose species richness is also known to climax in the photic zone (da Gama et al., 2014). Surface 'clotting' by fouling organisms is undesirable due to its potential to reduce algal fitness, by (1) tissue damage by mechanical anchoring and thallus penetration, (2) affecting nutrient and gas exchanges that take place through the mostly undifferentiated thallus surface, (3) affecting photosynthetic activity, and (4) affecting reproduction as the reproductive stages from macroalgae, such as gametes and different types of spores, are usually released from the seaweed's surface (da Gama et al., 2014; Gonzalez & Goff, 1989). Indirect effects include an increased drag with consequent tissue loss during extreme weather events and advanced tissue damage due to organisms grazing on the epibionts (the so called 'shared doom' effect, proposed by Dixon et al., 1981). As a consequence, some macroalgal taxa have evolved to respond to herbivory and competition by synthesizing "a pool of defensive

metabolites that can vary among individuals within a population, but also temporally and/or spatially" (e.g., Andras et al., 2012; Pezzolesi et al., 2021; Rasher and Hay, 2010; Rasher et al., 2012). Allelopathy, as initially introduced by Molisch (1937), was defined as "the biochemical interaction, both stimulatory and inhibitory, between primary producers and/or microorganisms" (Table 1). The term itself stems from the Greek *allelon* (of one another) and *pathos* (suffering), mainly focusing on the deleterious effects from plants (Harlin and Rice, 1987). There has been considerable discussion of the concept and definition of allelopathy in the past (Muller, 1969; IAS, 1996; Rice, 1984). Nowadays, in literature, allelopathy is predominantly regarded to be a distinct strategy to either eliminate or to deter coexisting competitors and/or predators (e.g., Budzałek et al., 2021; Gomes et al., 2017; Śliwińska-Wilczewska et al., 2021). In this review, we will interpret the term allelopathy in the light of the negative effect(s) of bioactive compounds secreted by macroalgae to target other macroalgae/other competing organisms.

In aquatic ecosystems, so far, allelopathy has been investigated more (profoundly) in freshwater habitats compared to the marine environment (Ben Gharbia et al., 2017; Erhard, 2006; Macías et al., 2008). Nevertheless, as of late, research on allelopathy in macroalgae has increased profoundly. Therefore, Budzałek et al. (2021) reviewed allelopathy in macroalgae and its repercussions for coexisting animals, mainly focusing on herbivory, but animal competitors were included as well. Budzałek et al. (2021) mainly focused on listing the allelopathic macroalgae species, their distribution, and the affected animal species, excluding other potentially affected evolutionary groups. While their review touched upon the identity of some of the allelochemicals, a profound review on these compounds, their mode of action, as well as their production, is currently missing. Hence, the aim of the current article is to put forward the ecological roles and chemical diversity of (putative) allelochemicals produced/secreted by macroalgae in marine environments that affect the full range of aquatic competitors (but

excluding herbivory). We aimed to (1) characterize the diversity of (putative) allelopathic macroalgae and their target organisms, (2) outline the temporal and geographical distribution of publications studying allelopathy in seaweed, (3) summarize the incentives/relevance of studying allelopathy in seaweed, including their economic potential, (4) describe the commonly used methods for the study of macroalgal allelopathy, as well as their limitations, (5) compile information on the identity of the putative allelopathic compounds and their potential mode of action, and (6) to obtain a better understanding of the influence of biotic and abiotic parameters influencing allelopathy production and/or sensitivity.

Table 1: Terminology and definitions used throughout the text

Biochemical compound	A carbon-based compound found in living organisms
Bioactive compound:	A substance with a biological activity or with the ability to modify or regulate one or more metabolic processes in a living organism, tissue or cell
Algicidal compound	A compound killing or preventing the growth of algae
Allelochemical	Bioactive compound, produced by either plants, macro/microalgae, bacteria, or fungi that impact the growth, physiology and/or development of other organisms
Allelopathic compound	Synonym of allelochemical
Allelopathy	Any process in which bioactive metabolites secreted by an organism affect the development of another species
Antifoulant	A substance that prevents, reduces, or eliminates biofouling
Epibiont	An organism that lives on the surface of another organism
Basibiont	An organism that is host to an epibiont.

2. Methods

Literature search

In accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) recommendations (Moher et al., 2009), we searched two major databases to collect relevant peer reviewed papers. We searched both Google Scholar and Web of Science using all possible combinations of the search terms “allelopathy”, “allelochemicals”, “antifouling”, “seaweed”, “macroalgae”, “Rhodophyta”, “Chlorophyta”, “Phaeophyta”, and “Phaeophyceae”

from 29 June to 06 July 2022. We used the search criteria "all topics" to search Web of Science, returning in total 203 different articles (101 were deemed relevant and collected) and "in title" was used to search Google Scholar, returning 540 articles (39 additional relevant papers were collected, that were not yet found in the WoS database). We also scouted the literature that was cited in the acquired articles for appropriate publications (27 additional articles collected). To be included as relevant in our analyses, each article must have been an original, peer reviewed research study, reporting used methodology as well as the data analysis. Studies were only taken into consideration if the authors physically exposed a target organism to macroalgae in any form and if that target organism experienced a significant negative growth/metabolic impact. Studies that cannot fully claim allelopathy, due to incomplete investigations, or lack of control over confounding factors, were included in the meta-analysis. In such cases, the responsible compounds are referred to as either putative allelopathic or simply bioactive in this review. Studies that reported chemicals produced by macroalgae as a predator defence were excluded, as this is out of the scope of this review. We also excluded publications that examined the effects of allelochemicals on non-marine target organisms (i.e., terrestrial or freshwater species), as this is out of the scope of this review. In contrast, studies focusing on effects on pelagic target taxa (i.e., phytoplankton) were not excluded as allelopathic bentho-pelagic interactions due to competition for light and/or nutrients can take place, especially in coastal areas. The applied search criteria yielded a total assembly of 167 articles, which we screened for the following information: publication year, study location, the allelopathic and target taxa, the used exposure method(s), used bioassay(s), the mitigation of potential confounding variables, the identity of the allelochemicals, and the used methodology to isolate and identify the chemical compound.

Data analysis

All retrieved studies were summarized and relevant data (described above) was collected in one data table (Table S1). Figures were produced using the package ggplot2 (Wickham, 2009) in R v4.1.1 (R Core Team, 2017).

3. Results

In total, 167 research articles were collected, of which majority of the studies (n =80, 47.9 %) reported putative allelopathy present in at least one red seaweed species, followed by 74 brown macroalgae and 61 green macroalgae studies (44.3 % and 36.5 %, respectively; Table S1, S2). More statistics about country of origin, tested macroalgae and target taxa can be found in supportive information (Fig. S1-S4). The results sections is outlined as follows: we first discuss which methods have been used to investigate allelopathy in macroalgae. Subsequently, we discuss the production of allelochemicals and how the environment can influence their production or effectiveness. Finally, we focus on the identity of allelochemicals and discuss their mode of action, prior to highlighting the different incentives to study this phenomenon.

3.1. Commonly used practices to study allelopathy

Several research methods are required to identify and confirm the presence of allelopathy in macroalgae and their impact on competitors. We distinguish seven different methods for observing macroalgal allelopathy, ranging from field experiments to exposure to thalli extracts (Fig. 1).

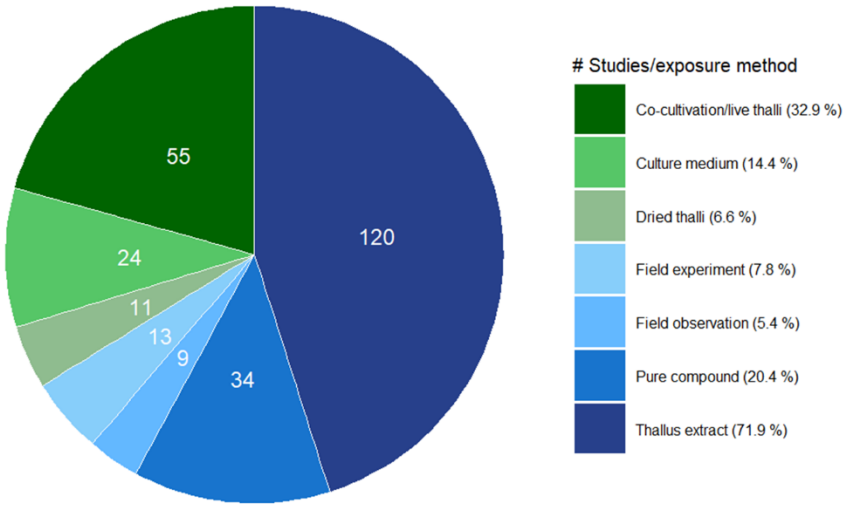

Figure 1: Pie chart of absolute number of studies using a certain investigation method. Relative numbers (percentages) are added to the legend.

- Field observations relate to observing the co-occurrence (or absence) of the competitors in the natural environment. For coral-algae interactions, studies focus on contact zones between the coral and the seaweed thallus, and whether bleaching has occurred or not (e.g., Rickert et al., 2015, 2016; Slattery and Lesser 2014; Vermeij et al., 2011). Field observation studies, on their own, are not sufficient to demonstrate the presence of allelopathy, as several confounding variables might be at play (cf. more profound discussion in section 3.2).
- A second group of studies have used field experiments to demonstrate allelopathy (found in 7.9 % of the collected studies). For example, investigating coral-seaweed interactions, several studies have manipulated contact between coral and macroalgae in the field and investigated bleaching and mortality rates after exposing the corals to either live macroalgal tissue (e.g., Bonaldo and Hay, 2014) or their extracts, embedded in phytagel disks (e.g., Rasher and Hay, 2010; Slattery and Lesser, 2014). In the recruitment plate method, the impact of seaweed on other organisms is studied by observing the settlement and survival rate of target taxa on specially placed grids or substrates, positioned in the field.

- Third, 55 publications (33.3 %) have used co-culturing experiments or microcosms to identify allelopathy by focusing on effects on the target organism after exposure to live thalli of the potential allelopathic species (e.g., in Ben Gharbia et al., 2017; Svensson et al., 2013; Wang et al., 2016, 2017b). Similarly, co-cultivation on its own is usually not sufficient to demonstrate allelopathy due to confounding variables that might be responsible for the observed effect (see discussion in section 3.2).
- Some studies choose to work with dried thalli rather than live thalli, hereby avoiding some of the confounding variables mentioned in section 3.2 (6.7 % of total studies; Table S1).
- The fifth method includes investigating the effect of the macroalgae culture medium (filtrate) on the target organism (applied in 14.5 % of the collected studies). Here, the macroalgae are cultured for a well-defined time period in the culture medium that, subsequently, will be used for exposure, after correcting the nutrient concentrations and filtering (for example with an autoclaved membrane filter) to avoid bacterial contamination (e.g. Wang et al., 2006). The culture medium is added at the beginning of the experiment (initial addition) or added intermittently (semi-continuous addition), when part of the culture medium is removed daily and replaced with an equal volume of macroalgal medium filtrate, re-enriched with nutrients (e.g. Wang et al., 2007). The latter method ensures the effectivity of an allelochemical, as it controls for potential degradation of the compound, as its half-life depends on its chemical structure.
- In the most-used method (exploited in 71.5 % of the studies analysed), the effect of macroalgal extracts (of either fresh or dried thalli or thallus powder) on the growth, survival, physiology and/or development of the competitors are analysed (e.g., Fu et al., 2022; Pansch et al., 2009; Zhang et al., 2021; Table S1). Depending on the test organism, these extracts can be tested either dissolved in the test medium, in phytagel disks, in

medium in a petri dish. While, ideally, thallus surface extracts should be tested, most studies have used whole cell extracts to test for effects on the tested target organism.

- Finally, 20.6 % of the studied have tested pure isolated compounds (from extracts) for its allelopathic properties (Table S1).

While most of the collected studies in this review have illustrated the inhibiting activity of a compound through one or more of the methods described above, few studies have demonstrated that the inhibitory activity is ecologically relevant, i.e., that the algal metabolites are present at the algal surface, or released in the surrounding water at concentrations that are deterrent to naturally occurring foulers (e.g., Dworjanyn et al., 2006; Schmitt et al., 1995). For example, a majority of studies have been testing crude seaweed extracts rather than surface extracts or have not considered confounding factors (as elaborated on in section 3.2). These studies imply the presence of allelochemicals but overlook other factors, implying to be careful in attributing allelopathic properties; they could be merely bioactive. In most cases, more research is thus required to truly confirm the presence of allelopathy. Only a quarter of studies (42) addressed the challenges mentioned above in their designs. Hence, we decided to not cover the less relevant studies in terms of ecology in this review, they are only considered in the statistical summaries in the supplementary information and in the discussion of the chemical identity of the allelochemicals.

3.2. Confounding variables and other challenges studying allelopathy in macroalgae

Observing allelopathy among aquatic organisms in a natural setting provides the best evidence of these negative interactions effectively occurring in nature. Yet, investigating allelopathy in the field is often a challenge, as several environmental factors can mask allelopathic effects (Legrand et al., 2003). Hence, several studies attempted to characterise allelopathic interactions in co-cultivation experiments, in a controlled system (i.e., controlled light and illumination

period), where confounding variables can be actively controlled. If the identity of the allelochemical is not known, reductions in growth, or reduced physiological status, could be attributable to multiple factors. Below, we briefly discuss these confounding variables and we provide several recommendations and suggestions to take these factors into account in future studies.

First, in co-cultivation experiments, a decline in macroalgae/microalgae growth can be anticipated, due to significant nutrient consumption and competition, as seaweed are known to consume a substantial amount of nutrients (Bambaranda et al., 2019; Neori et al., 2004; Patil et al., 2020). By actively monitoring the nutrient flux during the experiment, studies obtained a better insight in the nutrient flow and whether nutrients became depleted during the experiment or not (e.g., Accoroni et al., 2015; Nan et al., 2014; Table S1). Other studies (e.g., Nan et al., 2014; Patil et al., 2020) tackled this problem by periodically replacing part of the culture medium with an equal amount of fresh medium.

Second, shading by (floating) macroalgal thalli may affect the growth of other photoautotrophic species (White and Shurin, 2011). Several studies (e.g., Ma et al., 2017; Nan et al., 2014; Patil et al., 2020; Table S1) mention that light limitation was unlikely in their experiment as the macroalgae were always at the container's bottom, with the light source above the flasks. Additionally, Tang et al. (2011) applied a PAR sensor to measure and confirm that light intensity did not fluctuate significantly among the different treatments.

Third, studies that investigate the contact between the seaweed surface and the contact organism (such as corals), should apply a plastic model or another algal mimic to control for shading, abrasion, fractures, and the presence of a cable tie/other objects to connect the organisms (e.g., Andras et al., 2012; Del Monaco et al., 2017; Rasher et al., 2011; Slattery and Lesser, 2014; Table S1).

Furthermore, macroalgae can compete with co-occurring macro/microalgae through pH alteration (Green-Gavrielidis et al., 2018; Sylvers and Gobler, 2021). Seaweed may increase the pH of the test medium in which it is cultivated, by rapid photosynthesis and, thus, reducing pCO_2, making it unsuitable for other organisms to grow (Hansen, 2002; Patil et al., 2020; Sylvers and Gobler, 2021). For instance, *Ulva intestinalis* has been shown to increase the pH of rock pools to above 10, a pH level where other macroalgae can no longer utilize the enzyme carbonic anhydrase to convert HCO_3^- to CO_2 for use in photosynthesis, hence becoming carbon limited (Björk et al., 2004). Hence, it is crucial that pH is regularly monitored in co-cultivation studies (e.g., in Green-Gavrielidis et al., 2018; Jin et al., 2003; Nan et al., 2014; Sylvers and Gobler, 2021; Tang et al., 2011; Table S1) to ensure the observed effects are not the result of unsuitable pH values of the test media. Few studies actively maintained pH until termination of the experiment by adjusting the pH accordingly (e.g., Accoroni et al., 2015; Benitt et al., 2022). In their experiments, Patil et al. (2020) describe how they provided a continuous supply of air to the culture medium via bubbling to counterbalance the observed pH increase.

Finally, several publications have also demonstrated that high levels of epiphytic and biofilm forming bacteria can grow on the surface of the seaweed thalli and that some of these species have growth-inhibiting and algicidal properties as well (Imai et al., 2002, 2006; Inaba et al., 2017, 2020; Mayali and Azam, 2004). To prevent strong impact from these bacteria, several studies suggested/took precautionary measures, such as liberal rinsing of the thalli with sterile seawater or physically wiping of the macroalgae, yet the most suitable option is the inclusion of an antibiotic (mixture) in a pre-treatment step (e.g., Benitt et al., 2002; Denboh et al., 1997; Jeong et al., 2000; Jin et al., 2003; Tang et al., 2011; Table S1). Antibiotics that have been applied in the investigated studies include penicillin, streptomycin, chloramphenicol, ampicillin, kanamycin, polymixin, neomycin or a combination/ mixture of some of these. Tang

et al. (2011) performed periodic DAPI-staining of cultures to confirm the absence of bacteria in their cultures.

If the results in co-cultivation systems cannot be entirely attributed to the effects of nutrient depletion, pH elevations, light limitation, or algicidal bacteria, much of the inhibiting activity observed is usually attributed to allelopathic metabolites secreted by macroalgae (e.g., in Patil et al., 2020). Ideally, such findings should be confirmed by including other experiments and/or the identification of the responsible compound(s). Finally, field studies should confirm whether the observed interactions occur in a natural setting under the influence of natural processes, such as advection, mixing of the water column, turbulence, etc., or if they only take place in controlled conditions, i.e., in stagnant water in the lab.

3.3. Sites of production, transport and storage of allelochemicals in macroalgae

Excretion or contact bound allelopathy?

One important reason for our limited apprehension of allelopathy involving macroalgae is related to the scarcity of knowledge on the localization of allelochemicals on/in seaweeds and the potential processes that transport these compounds to the thallus surface. Bioactive compounds may have various ecological functions (e.g., simultaneously functioning as an anti-herbivore and an antifouling agent, as for example found in Schmitt et al., 1995), however in some instances they exert a distinct functional role, and in those instances they may need to be differentially distributed on/in macroalgae, according to their role. Herbivory deterrence can be achieved by storing compounds within the seaweed, as herbivores encounter these metabolites upon biting and ingesting entire thalli (Lane et al., 2009; Schmitt et al., 1995). However, in order to be effective as defensive compounds, it would be more efficient if they are deployed on the thallus surface, as this is the interface where interacting organisms, such as settling larvae, come into contact (Andras et al., 2012; Nylund et al., 2007; Lane et al., 2009; Schmitt

et al., 1995; Steinberg and de Nys, 2002). Based on reviewed literature, two main types of allelopathic mechanisms in macroalgae are suggested. First, seaweed can target competitors and/or epiphytes by direct contact (i.e., through surface-associated compounds). The chemistry of allelochemicals is most likely hydrophobic in nature, and thus non-polar, with the purpose of maximizing its concentrations at/near host surfaces and avoiding swift dissolution (A Abdul Malik et al., 2020; Steinberg and de Nys, 2002). The dominance of fatty acids/lipids as allelochemicals (see below) makes sense in an evolutionary and energetic context given the potential of the marine environment to dilute water-soluble compounds (Rasher and Hay, 2010). Secondly, macroalgae could produce a sufficient amount of polar bioactive metabolites, which is subsequently excreted into the water and reaches target taxa with an effective, active concentration (A Abdul Malik et al., 2020; Gross, 2003). Compared to terrestrial plants, seaweed have thalli without stomata, with less tight cell connections, and with a reduced (or even absent) cuticle (Hutchinson, 1975), likely facilitating a direct release of hydrophilic, water-soluble metabolites into seawater. However, both dilution and short half-life of these type of allelopathic agents are major challenges, hence requiring greater concentrations of macroalgal allelochemicals (Fong et al., 2019). Solely based on polar properties, it is challenging to ascertain whether a single metabolite could exert an allelopathic function at the macroalgal interface (A Abdul Malik et al., 2020). Physicochemical interactions between the allelochemical and the seaweed surface (e.g., through ionic interactions or hydrogen bonds) could promote or restrict its diffusion into the environment, as suggested by A Abdul Malik et al. (2020). The storage location, the site(s) of production, as well as the disposition among external and/or internal tissues of most allelochemicals has not been established yet, but a couple of bioactive compounds have been determined to specifically be present on the thallus surface of seaweed (e.g., Andras et al., 2012; Dworjanyn et al., 1999; Lane et al., 2009; Schmitt et al., 1995). For instance, Schmitt et al. (1995) demonstrated that larvae of the bryozoan *Bugula*

neritina were not affected by water from near the brown macroalga *Dictyota menstrualis* and did not avoid contacting *Dictyota*, relative to another substrate. However, the larvae would not settle on *Dictyota* following contact. These findings suggested the presence of a deterrent hydrophobic/lipophilic metabolite located on the thallus surface of *Dictyota*, i.e., the compounds pachydictyol A and dictyol E, that were not released into the water. Furanones from the rhodophyte *Delisea pulchra* were also quantified at the algal surface and studied as antifoulants towards a range of target organisms (Dworjanyn et al., 1999, 2006). Finally, Rasher and Hay (2010) found lipophilic extracts from a number of seaweed species, including the brown macroalga *Lobophora variegata*, caused significant coral bleaching, while this was not the case for the hydrophilic metabolites extracted from the, respectively, green and red macroalga *Chlorodesmis fastigata* and *Galaxaura filamentosa*. Up to now, all demonstrated allelopathic interactions between seaweed and corals depend on the transfer of compounds via direct contact (via tissue bound allelochemicals), rather than via transmission across seawater (de Nys et al., 1991; Rasher and Hay, 2010). This suggests that contact generated effects, rather than proximity alone, and that their allelopathic agents are lipid soluble rather than hydrophilic in nature.

Together, these findings seem to confirm the importance of direct contact, which would be advantageous for lipophilic allelopathic compounds. Yet, in several studies (e.g., Jin et al., 2005; Nan et al., 2008; Nelson et al., 2003; Patil et al., 2020; Wang et al., 2007, 2012; Xu et al., 2012), isolated live thalli (co-cultivation), growth medium filtrates, dried thalli, and aqueous extracts of thalli all dwindled the growth of harmful algal blooms, suggesting that direct contact is not always required to cause the observed inhibitory effects. The possibility of growth inhibition by direct contact with the seaweed specimen itself can be ruled out by carrying out isolation co-culture assays (e.g., Jin et al., 2003).

Storage and transport processes

Whether secreted or surface bound, currently little is known as well about the mechanisms/processes related to storage and transport to the seaweed's thallus surface. In seaweed, bioactive metabolites are potentially stored in internal structures inside the thallus and not distributed to its surface, are potentially made in inner glands but secreted into the exterior via a connection pore, or can be allocated to the thallus surface via an unknown route (Andras et al., 2012; de Nys et al., 1998; Dworjanyn et al., 1999; Lane et al., 2009; Paul et al., 2006). Bioactive compounds that come to the surface and that have been observed to act as allelochemicals, have been found to be stored within several types of specialised structures associated with the thallus surface: secretory trichomes; epidermal gland cells (or vesicle cells), in which a storage vesicle preoccupies almost the entire cellular space (Dworjanyn et al., 1999; Steinberg and de Nys, 2002), physodes, i.e. membrane-bound vesicles from brown macroalgae (Schoenwaelder, 2002), and *corps en cerise* or ''cherry bodies'' (CC), which are spherical, reniform to claviform, refractile intracellular inclusions mostly occurring in the cortex of red algae species of the genus *Laurencia* (Feldmann and Feldmann 1950; Salgado et al., 2008; Young et al., 1980). For instance, *Laurencia dendroidea* was shown to store halogenated metabolites in the *corps en cerise* and to transport these through vesicular traffic to the seaweed's surface in response to epiphytic bacteria (Salgado et al., 2008, Paradas et al., 2010). The production and transfer of most bioactive metabolites remain unclear however. For example, Paul et al. (2006) reported about the presence of stalk-like structures in *Asparagopsis armata* and argued that these could function as a possible mechanism to transport allelochemicals to the surface of the thalli. The red alga *Bonnemaisonia asparagoides* has multitudinous specialized gland cells on its thallus's exterior, in which it likely stores its antibacterial metabolites to release them to the thallus surface, as suggested by Nylund et al. (2010).

Reis et al. (2013) were able to demonstrate that both microfilaments and microtubules play a pivotal part in the vesicle transport from the *corps en cerise* to the periphery of the cells for subsequent exocytosis of the secondary metabolites in *Laurencia dendroidea*, as well as the anchoring and positioning of the CC's themselves to the cell's periphery. Via time-lapse video microscopy, exocytosis of these vesicles in the cortical cells of *L. obtusa* through the plasma membrane to the inner cell wall was observed by Salgado et al. (2008). They argued that exocytosis transfers the bioactive metabolites to the seaweed's exterior and that fluctuations in this process might result in varying concentrations of the metabolites on the seaweed's exterior. Next to allowing the regulation of metabolite exudation, another explication for the energetic investment in storage room (e.g., the *corps in cerise* in *Laurencia*, the physodes of brown seaweeds, the gland cells in *Delisea pulchra*) and the transport mechanisms into the apoplast, might be the autotoxicity of allelochemicals, as compartmentalization and transport can abate the concentration of the metabolites in the cytosol, lessening cytotoxicity (Shitan, 2016).

The necessity to differentiate between whole tissue extracts and thallus surface extracts

Most of the investigated studies used crude-extracts of entire macroalgae to screen directly for allelopathic activity (Table S1). The use of crude extracts of fresh thalli or dried powder theoretically might have an advantage over the direct application of fresh tissue, as concentrations can be controlled for as well as eliminating the discussed confounding variables (section 3.2). In contrast, few studies have quantified the naturally occurring concentrations of allelochemicals on seaweed surfaces or have investigated the variation between surface-associated metabolites and those held within the seaweed's tissue. Yet, examining the localization, storage as well as the exudation mechanisms of allelochemicals is required to enhance our apprehension of the ecological significance of these metabolites (Nylund et al., 2007), especially from the exposure point of view. Although the crude extract procedure provides an insight in the broad-spectrum activity of bioactive compounds, the natural transport

mechanisms, as well as the responsiveness of ecologically relevant species to the secreted bioactive metabolites remain mostly undisclosed (Dworjanyn et al., 2006; Nylund et al., 2007). The whole thallus extraction approach risks to overestimate the defence capability of the studied macroalga, and the absence or presence of spatial allocation could significantly change the interaction outcome between competitors (Andras et al., 2012; Nylund et al., 2007; Steinberg and de Nys, 2002). If metabolites occurring within macroalgae are extracted and tested as if they were present on the macroalgae, then their true ecological effects are likely misrepresented (Longo and Hay, 2017). Few studies have explicitly tested this, and concluded that the inhibiting effects of whole thallus extracts were inadequate predictors of the inhibiting abilities of the tested macroalgae. For instance, elatol concentrations, obtained from total (lipophilic) tissue extracts of the rhodophyte *Laurencia obtusa* did suppress herbivorous and fouling activity significantly, while surface concentrations did not (da Gama et al., 2002; Pereira et al., 2003; Sudatti et al., 2008). Nylund and Pavia (2003) investigated the effects of lipophilic crude extracts obtained from four red macroalgae species on the settlement behaviour of cyprid larvae of the co-existing barnacle *Balanus improvisus* using different approaches. First, they found that larval settlement was strongly inhibited at presumably ecologically relevant surface concentrations, based on surface area/dry weight ratio estimations, for all four different seaweed species. These findings were then compared with a settlement preference experiment, in which was tested whether the cyprid larvae rather settled on algal surfaces or on control surfaces. The thallus surface of only one of the four tested seaweed species, *Chondrus crispus*, significantly hampered settlement in the preference experiment, while in one other species there seemed to be a settlement stimulation. Nylund and Pavia (2003) argued that these contradicting results likely are the consequence from the extraction of internal metabolites, that usually do not occur on the thallus surface. Longo and Hay (2017) performed several field experiments to assess the impacts of (1) lipid-soluble extracts from seaweed surfaces of multiple species, next to (2) total

lipid-soluble extracts from both internal and external seaweed tissues on the coral *Pocillopora verrucosa* and its symbionts. Extracts of the brown seaweed *Dictyota bartayresiana,* the green macroalga *Chlorodesmis fastigiata* and the red macroalgae *Amansia rhodantha* and *Asparagopsis taxiformis*, were found to suppress coral photochemical efficiency (Longo and Hay, 2017). However, they also found that the total lipid-soluble extracts of these species were not more toxic compared to the surface-only extracts, even though the concentrations of the total lipid-soluble extracts exceeded the surface-only extracts notably. Together, these findings suggest that bioassays with entire tissue extracts can be biologically meaningful but are also less ecologically relevant compared to the surface extracts. According to Paul et al. (2006), the delivery of a compound to the macroalgal surface and the inhibiting efficiency of the metabolite on the thallus designate important criteria that differentiate a natural antifoulant from potential bioactive metabolites. Hence, we recommend that future bioassays are preferably performed with the more simple, less concentrated seaweed surface extracts, and thus more relevant in terms of ecology. Several studies have already demonstrated allelopathy based on testing with relevant surface concentrations of the putative allelopathic agent. For example, surface-extracted compounds derived from the brown macroalgae *Fucus vesiculosus* and *F. serratus* were shown to deter the settlement of several micro- and macrofoulers (Rickert et al., 2015, 2016), whereas surface extracts generated from *Gracilaria vermiculophylla* were found to deter epiphytic, filamentous red macroalgae (*Ceramium* spp.) as well as benthic diatoms (Wang et al., 2017b).

There is to date no standardised method for testing ecologically relevant concentrations of surface compounds against fouling activity or any other type of competition. Most studies have employed the "swab" or "hexane dip" method to investigate undamaged biotic surfaces (de Nys et al., 1998; Nylund et al., 2005, 2007, 2010; Schmitt et al., 1995). The "hexane dipping" method, developed by de Nys et al. (1998), allows a quantitative extraction of hydrophobic

metabolites from the surface of the thalli without (visually) disrupting cells by dipping the algae in hexane for 5 to 40 s at room temperature. Several studies (e.g., A Abdul Malik et al., 2020; de Nys et al., 1998; Nylund et al., 2007), have used epifluorescence microscopy to clearly distinguish intact cells from lysed cortical cells of the macroalgae thalli after exposing (dipping) them to different solvents, such as dichloromethane, methanol, n-hexane, and dichloromethane/methanol (1:1), without any staining. In the study by Othmani et al. (2016), several dyes were tested to confirm the viability of cells from the brown alga *Taonia atomaria* after applying the 'dipping method' described above. More rigorous extraction methods using either other solvents, or longer extraction times in hexane (> 50 s), were found to cause significant visible cell damage (A Abdul Malik et al., 2020; de Nys et al., 1998; Nylund et al., 2007). However, while these procedures confirm whether the cell membrane remains intact after extraction or swabbing, they do not confirm whether or not a transfer of intracellular metabolites occurred without a visible cell disruption (Andras et al., 2012). Moreover, the dipping method only allows incomplete extractions of surface compounds, hence, likely underestimating the true allelochemical concentration (Wang et al., 2017a). These techniques have thus a limited ability to quantify metabolite distribution or concentration and are mostly valuable for confirming whether the bioactive compounds occur on the thallus surface.

Advances in imaging mass spectrometry have made it possible to analyse the chemistry of natural surfaces in their original state. Some studies have employed desorption electrospray ionization mass spectrometry (DESI-MS) to localise and measure bioactive metabolites on the thallus surfaces (Andras et al., 2012; Lane et al., 2009; Nyadong et al., 2009), illustrating the potential of this technique to determine the existence and concentrations of allelopathic metabolites on thallus surfaces. For example, Andras et al. (2012) used DESI-MS to visualise and quantify the metabolite neurymenolide A on the surface of the red alga *Phacelocarpus neurymenioides*, which was found to induce coral bleaching on natural colonies of *Porites rus*

444 in a field experiment. In conclusion, improving our comprehension of where compounds occur,
445 and in which concentrations and conditions, enlightens us about the metabolite's ecological
446 function and is critical for the design of ecological representational experiments (Andras et al.,
447 2012; de Nys et al., 1998; Lane et al., 2009).

448 *3.4 Biotic and abiotic factors affecting allelopathic behaviour and effectivity*

449 Abiotic conditions such as temperature, salinity, irradiance, UV radiation, pH, and nutrient
450 availability vary continuously in aquatic ecosystems and these fluctuations have a considerable
451 impact on the seaweed's growth, development and physiology (Gross, 2003; Jin et al., 2013).
452 The environment might influence allelopathic effectivity in at least three different ways: (1) the
453 production of the chemicals, (2) their bioavailability and (3) how they affect the target
454 competitor, which itself might be more sensitive as well to the allelopathic substance under
455 different circumstances (Gross, 2003).

456 Under conditions of environmental stress, a lower investment of resources and/or energy into
457 defence and increased investments into maintenance is predicted by the environmental stress
458 theory (Cronin, 2001; Pansch et al., 2009). The theory presumes that defences are costly, and
459 thus are actively reduced under resource limitation and environmental stress, yet these
460 assumptions have rarely been tested in experimental studies (Dworjanyn et al., 2006).
461 Noteworthy, Rasher and Hay (2014) found that competition with corals induced increased
462 allelochemical content in the seaweed *Galaxaura filamentosa*. Yet, they also observed the
463 seaweed experienced significantly reduced growth and increased palatability to herbivores
464 (because of reduced chemical defences). As they did not observe these patterns in non-
465 allelopathic species, it illustrates a complex coral-seaweed-herbivore interaction, with different
466 defense strategies against herbivores and competing corals. In terrestrial plants, there is strong
467 evidence that exposure to environmental stress induces the production of allelochemicals

(Muzell Trezzi et al., 2016). As chemical defense production can be adapted (regulated) according to the seaweed's need, it could be assumed that the production would fluctuate temporarily in temperate latitudes as both environmental factors, as well as biotic factors, such as fouling pressure, undergo seasonal shifts (Saha and Wahl, 2013). Hence, macroalgal defence intensity should thus either fluctuate (1) with abiotic factors controlling the energy resources, (2) with defence demand, such as fouling pressure, or (3) with both (Rickert et al., 2015). Despite common recognition of these impacts, data on allelopathic dynamics in marine ecosystems are restricted (Table S1; Gross, 2003). Producing defensive metabolites can fluctuate in several ways: seasonal or temporal, spatial and/or geographical, within populations (density dependent), or even within different parts of the thallus. Hence, several studies, mainly on temperate macroalgae, have recorded seasonal variability in antifouling activity (e.g., Hellio et al., 2004; Rickert et al., 2015; Saha and Wahl, 2013). The robustness of spatio-temporal patterns observed in the defence strength of several species, suggests that variation in allelopathy strength is not random and likely regulated. If so, the mechanistic foundation for such regulation, as well as its drivers remain to be identified and requires further research. In this section, we provide and discuss, non-exhaustively, a few studies in which biotic and abiotic factors have been suggested to affect either allelochemical production as well as sensitivity towards the allelochemical.

The influence of genetic factors

Variation in bioactive metabolite content in different seaweed populations has been documented in a number of studies (e.g., Koivikko et al., 2008; Pereira et al., 2004; Vallim et al., 2005). These fluctuations might be interpreted as resulting from genetic differentiation between populations and/or are the response to local changes in the environment, as described above, possibly affecting allelochemical activity. For example, Plouguerné et al. (2010) reported spatial variation of the antifouling activity of *Sargassum vulgare*, collected at different

493 sites along the coast of Rio de Janeiro, Brazil. Similarly, Saha and Wahl (2013) observed anti-
494 settlement activity of eight biofilm-forming bacteria after exposure to surface extracts of *F.*
495 *vesiculosus* and found a consistent and compelling difference between locations throughout the
496 year. In contrast, Jeong et al. (2000) found no regional and seasonal variation (collected
497 bimonthly from five different locations around Korea) in the algicidal activity of *Corallina*
498 *pilulifera*. This research area will likely progress with increased efforts in understanding the
499 genetic structure of macroalgae populations as well as the characterisation of biosynthetic
500 pathways, enzymes and genes in control of the production of the allelopathic agents.

501 *The influence of light intensity and irradiance*

502 The distribution pattern of light is rather complex in aquatic systems, with both low and high
503 light intensities limiting photosynthetic efficiency, either by insufficient energy input or by
504 photo-inhibition (Cade-Menun & Paytan, 2010). Light can impact allelopathic effectiveness at
505 different levels. First, light intensity is presumed to impact photosynthetic rate and, thus,
506 seaweed physiology, e.g., variation in protein and lipid content (e.g., Cade-Menun & Paytan,
507 2010; Jin et al., 2016). For example, assuming that fatty acids (mainly PUFAs) are the bioactive
508 compounds of several seaweed species (e.g., Alamsjah et al., 2005, 2008; Jin et al., 2016; Tang
509 and Gobler, 2011), an increased concentration of these fatty acids under lower/higher light
510 intensities may increase the biocidal effect. Second, photochemical reactions of the allelopathic
511 agents under high levels of irradiance might improve or decrease their effectivity (e.g., through
512 photodegradation for example; Jin et al., 2016). Finally, also the sensitivity of the target
513 organism might be altered after exposure to different levels of irradiance and allelochemical
514 concentrations. However, study-based evidence for these hypotheses is rare. For instance,
515 Pansch et al., (2009) did not find an effect of light reduction on the chemically mediated
516 defences of *Dictyota kunthii* on the bioactivity against mussels.

517 *The influence of temperature, pH and ocean acidification*

Temperature and pH are key factors affecting the growth and physiological activities of all aquatic organisms. Similarly as to light intensity, temperature and pH changes might alter allelopathic efficiency and/or sensitivity. Valenti et al. (2010) proposed that pH can change the ionisation of metabolites, thereby influencing the lipophilicity, bioavailability, bioaccumulation and aquatic toxicity of allelopathic compounds. For example, Del Monaco et al. (2017) demonstrated that lipophilic, surface extracts of the brown seaweed *Dictyota cervicornis* were more harming to the coral *Acropora intermedia* when competing under CO_2 levels predicted to occur in 2050 and 2100, compared to current-day, ambient conditions. The extracts from two other tested macroalgae species *Chlorodesmis fastigiata* and *Amansia glomerata* were not more potent to the coral when grown under increased CO_2 conditions (Del Monaco et al., 2017). Several studies reported that the growth inhibitory efficiency of the examined seaweed species was modulated by temperature (e.g. Denboh et al., 1997; Jin et al., 2016; Wang and Tang, 2016; Table S1). For instance, Denboh et al. (1997) reported a temperature dependent growth inhibition of *Laminaria japonica* after exposure to rhodophytes *Corallina pilulifera* and *Pneophyllum zostericolum*, especially at higher temperatures. In a series of multivariate experiments, Jin et al. (2016) found that the growth inhibitory impact of *Ulva pertusa* on co-cultivated *H. akashiwo* was most potent at the second highest temperature tested (25°C) with, high irradiance (400 μmol m^{-2} s^{-1}) and high alkalinity (pH = 10).

Nutrient concentrations

Many macroalgae are often restricted in their capacity to concentrate and store nitrogen internally, depending on a continuous and prominent supply of nitrogen (Xu et al., 2012). *Ulva* spp., for example have a high surface area/volume ratio and exhibit high rates of nutrient uptake (Xu et al., 2012; Zhang et al., 2013). However, several species are capable of storing nutrients; e.g., the genus *Gracilaria* requires only pulse fertilisation, as it is capable of storing nitrogen, partly as the pigment phycoerythrin (Friedlander et al., 1996). Benitt et al. (2022) investigated

543 the co-effect of nutrient limitation and allelopathy in the inhibition of the blooming pelagophyte
544 microalga *Aureococcus anophagefferens* (Ochrophyta) by *Gracilaria* and *Dasysiphonia*. They
545 found that allelopathy effectivity depended upon nutrient conditions and that *A.*
546 *anophagefferens* was apparently more resistant to seaweed cultured in low nutrient
547 concentrations.

548 *Population densities*

549 More biomass in both natural and culture systems steers towards (more) nutrient limitation and,
550 hence, a fiercer competition (Gao et al., 2014). Multiple studies have indeed examined whether
551 higher macroalgal population densities/biomass impact allelopathic effectivity under fixed
552 light, nutrient and pH conditions (e.g. Lv et al., 2021; Patil et al., 2020; Tang et al., 2015; Wang
553 et al., 2013; Xu et al., 2012; Table S1). Benitt et al. (2022) described how red algae species
554 significantly reduced densities of the microalga *A. anophagefferens* in a dose-dependent way,
555 with the effectivity of each species depending mainly on both *A. anophagefferens* abundances
556 as well as the seaweed densities themselves. From the target point of view, Patil et al. (2020)
557 described how the inhibitory effects of the thalli of the red seaweed *Pyropia haitanensis* were
558 substantially reduced at higher initial microalgal cell densities of the bloom forming
559 *Skeletonema costatum* (Bacillariophyceae). Similarly, an attenuation in the inhibition of the
560 thalli of *Porphyra purpurea* on the growth of *A. anophagefferens* and *Karlodinium veneficum*
561 (Dinoflagellata) was noted after an elevation in initial microalgal cell abundance (Tang et al.,
562 2015).

563 *Tissue age and specialisation*

564 The age of the studied tissue can also have an impact on the concentration of the studied
565 allelochemical. For example, Andras et al., (2012) found that average concentrations of the
566 allelochemical neurymenolide A were more than double on older, bottom portions of the
567 sampled thalli compared to those of younger tissues in the blades of the rhodophyte

Phacelocarpus neurymenioides. This could hint that this macrolide is actively being sequestered in elder parts to defend the basal portions of the thallus that anchor the macroalga. Alternatively, it could suggest that the allelochemical accumulates over time in tissues, or that it is selectively transported to older parts of the thallus, as these parts have encountered long-term exposure to fouling organisms already (Andras et al., 2012). Additionally, Plouguerné et al. (2012) observed tissue specialisation in *Sargassum vulgare* in regard to its antifouling activity against the mussel *Perna perna*. They observed that polyphenol extracts from its pneumatocysts (air vesicles, providing buoyancy) had the highest antifouling activity, followed by the leaflets, while the reproducing structures, i.e. the receptacles, exhibited the least antifouling activity.

Predation

Several studies have examined the impact of predation on the production of allelopathic compounds or vice versa. Ensuing eight days of competing with the coral *Porites cylindrica*, the macroalga *Galaxaura filamentosa* elevated allelochemical production and was almost as double harmful to the coral (Rasher and Hay, 2014). However, owing to competition, *Galaxaura* itself experienced a reduced growth and a softening of its anti-herbivore chemical defence, followed by an increased palatability. Rasher and Hay (2014) concluded that *Galaxaura* secreted different chemical compounds to oppose/resist competitors versus herbivores. In some species, bioactive compounds are broadly defensive against a wide range of both consumers and competitors, circumventing the energy costs of herbivore deterrence as the same trait both provides herbivore deterrence/resistance, as well as offers competitive advantages (Schmitt et al., 1995). For instance, Schmitt et al. (1995) were able to demonstrate that diterpene alcohols, secreted by brown macroalga *Dictyota menstrualis*, are not only used to defend itself from predation by sea urchins and herbivorous fishes, but also serve as allelochemicals damaging the larvae of fouling organisms.

3.5. The identity of (putative) allelochemicals in macroalgae

Identification of the responsible allelochemical is a costly and time-consuming undertaking. Yet, 32.9 % (n = 55) of the investigated and collected studies have described at least one chemical, produced by either brown, green and red macroalgae, that significantly affects the biological activity of one or more target organisms (Table S3). In total, bioactive compounds have been identified in 57 different species so far, of which almost half of them brown macroalgae (n = 26, 45.6 %). In comparison to Rhodophyta (n =20; 35.1 %) and Ochrophyta, fewer compounds in Chlorophyta were described to date (n =11 species; 19.3 %), of which more than half belonging to the genus *Ulva* (Table S3). Remarkably, only twelve of these 55 studies (21.8 %) have truly identified an allelochemical with inhibiting properties via realistic exposure conditions (i.e., through ecologically relevant assays, as discussed profoundly below; Table S3). Hence, much effort still lies in the characterization of allelopathic compounds in macroalgae, as well as in distinguishing true allelochemicals from bioactive molecules.

According to their chemical structure, four major categories of algal synthesized bioactive compounds can be distinguished: (1) carboxylic acids/fatty acids and derivatives; (2) (poly)phenolic compounds; (3) terpenoid compounds; and one of its subgroups (4) sterols (Table 1). Fatty acids and derivatives were the most retrieved group of putative allelochemicals in our survey (59.6 %) in all three evolutionary lineages, including the regularly found hexadecenoic acid, octadecanoic acid and eicosatetradeconoic acid (Table 1). The occurrence of simple phenols, such as flavonoids, benzoic acids and derivates has been observed in all seaweed lineages, but brown macroalgae have been described to possess higher phenolic contents relative to green and red macroalgae (Aina et al., 2022; Cotas et al., 2020). In the searched literature, only terpenes/terpenoids with a growth inhibiting activity in brown and red macroalgae have been described so far (Table S3), even though terpenoids are occurring in all living organisms (because of their role in the respiration chain electron transport).

While it is important to investigate the structure and identity of $ allelochemicals, we should recall that allelopathic function might not depend exclusively on one key metabolite. Mixtures of compounds could act synergistically or additively, even when the pure compounds individually might not cause a visible effect (Blum, 1999).

3.6. The allelopathic mode of action

Although the inhibitory consequences of macroalgae extracts have been studied on a plethora of species, not much is known about their mechanisms of inhibition in the target taxon. Allelochemicals likely cause disturbances in range of physiological and developmental processes in target organisms (Ben Gharbia et al., 2017). To understand their mode of action as well as to establish the extent of harmful impacts on the neighbouring/target species, several investigations must conducted to elucidate the physiological mechanisms of allelopathy.

The inhibition of photosynthetic efficiency or functioning of competing primary producers is considered a very common mode of action among many photosynthetic organisms. Photosystem II (PSII), a membrane protein supercomplex executing the first reaction of the photosynthetic pathway, is most often identified as being the main target (Ben Gharbia et al., 2017; Gross, 2003). To examine photosynthetic energy conversion, the maximum quantum yield of PSII, i.e., a proxy for the efficiency of excitation energy capture, can be used to confirm the integrity and functioning of PSII and has been broadly used to analyse effects of stressors/stress on the target organism's photosynthetic activity (e.g., Baker and Rosenqvist, 2004; Gross, 2003; Table S1). For example, exposure to thalli of *Ulva rigida* caused a significant decrease in the maximum PSII quantum yield in several dinoflagellate species in a dose-response manner (Ben Gharbia et al., 2017). Ye et al. (2014) found that dried thalli of *Gracilaria lemaneiformis* mainly inhibited photosynthetic targets in *Scrippsiella trochoidea* (Dinoflagellata) with a reduction in the quantities and sizes of antenna chlorophyll, a decrease

642 in photochemical efficacy of PSII, obstruction of the electron transport chain, in addition to
643 damage to the oxygen-evolving complex. None of the studies were able to describe the initiating
644 event in the inhibition of the photosynthetic pathway. Nevertheless, considering the crucial
645 importance of photosynthesis photoautotrophs, a reduction in efficiency of the photosynthetic
646 apparatus, induced by an allelochemical, is a strong indicator of its phytotoxicity (Raniello et
647 al., 2007; Ralph, 2000).
648 Some authors have argued that (microalgal) cells could be triggered to produce reactive oxygen
649 species (ROS) and trigger oxidative stress by stimulation of allelochemicals (El-Darier et al.,
650 2021; Qian et al., 2009; Sun et al., 2012, 2021b; Xu et al., 2020; Zhang et al., 2021).
651 Allelochemicals might potentially cause an increase in oxygen free radicals (Gill and Tuteja,
652 2010). These radicals are usually involved in membrane lipid peroxidation, degradation of
653 chloroplasts and in the reaction with several macromolecules, subsequently disturbing cell
654 growth and proliferation (Gill and Tuteja, 2010). To evaluate the effect of α-linolenic acid,
655 identified in crude *Sargassum fusiforme* extracts, on antioxidant enzymes, Sun et al. (2021b)
656 measured superoxide dismutase (SOD) and peroxidase (POD) activity, two common
657 antioxidant enzymes of HAB species *Heterosigma akashiwo*. Their analyses showed that SOD
658 and POD activity gradually decreased under high α-linolenic acid concentrations. Similarly, the
659 activities of four oxidoreductases, including SOD, showed an initial increasing trend of activity
660 and then decreasing in the diatom *Skeletonema costatum* after cultivation with *S. fusiforme*
661 extracts as well (Zhang et al., 2021). According to the authors, these results can be interpreted
662 that the stress caused by low extract concentrations initially increased ROS levels in diatoms,
663 as well as that antioxidant enzymes were not capable of eradicating the excessive amount of
664 oxidation products, which in turn caused damage to the enzyme system. In other words,
665 excessive amounts of free radicals cannot be eradicated by the antioxidant system, which
666 potentially affects the activity of its enzymes in turn (Gill and Tuteja, 2010; Zhang et al., 2021).

667 Another allelopathic mechanism in macroalgae that has been suggested is associated with the
668 disruption of the osmotic pressure regulation, mainly by studies that found changes in the shape
669 and/or motility of exposed (micro)algal cells. For example, phlorotannins from phaeophyte
670 *Ecklonia kurome* made the cells of three red tide HABs lose their motility and almost all
671 microalgae became round in shape before expanding and bursting (Nagayama et al., 2003).
672 Nagayama et al. (2003) argued that interactions between phlorotannins and microalgal channel
673 proteins in their membranes played a pertinent role in the algicidal actions of phlorotannins,
674 consequently disturbing the control of osmotic pressure. Wang et al. (2013) speculated as well
675 that the allelopathic mode of action through which *Ulva pertusa* or *U. prolifera* inhibited the
676 growth of the dinoflagellate *Prorocentrum donghaiense* might be associated to a disrupted
677 cellular control of its osmotic pressure, because motility and modality of the microalgal cells
678 were limited by the allelopathic compounds as well. Wu et al. (2006) described that fatty acids
679 caused rapid permeability changes in plasma membranes, which are related to K^+ ion leakage,
680 and the resulting degradation of the organisation of the membrane. In the study of Oh et al.
681 (2009), cells of the affected organism first displayed swelling and then lysed during exposure
682 to the fatty acid EPA. Despite these finding based hypotheses, the mode of action of most
683 allelochemicals remains unclear. To enhance our understanding of underlying mechanisms of
684 action, further research is required.

685 ### *3.7. Incentives and relevance of studying macroalgal allelopathy*

686 The interest in allelopathy among aquatic organisms is growing for several reasons. Each of the
687 main incentives will be discussed briefly below.

688 #### *The role of allelopathy in shaping and structuring marine communities*

689 The character and intensity of competitive interactions between organisms influence the
690 functioning and structure of any community (Maggi et al., 2012; Nabivailo et al., 2014; Paine,

1990). Allelopathy among primary producers has been reported to have a considerable impact on the structure and succession of an aquatic ecosystem (e.g., Getachew et al., 2017; Gross, 2003; Jin et al., 2016; Kakisawa et al., 1988; Kim et al., 2004; Green-Gavrielidis et al., 2018; Slattery and Lesser, 2014). Macroalgal communities are usually dominated by species with high competitive abilities under certain biotic and abiotic settings (Carpenter, 1990). This ability can either be (1) rapidly adapting to the limitations in resources and exploiting them effectively, making the resources inaccessible for their competitors (i.e., exploitative competition), or (2) influencing the competitor's physiology through allelopathy (i.e., interference competition) (Maggi et al., 2012; Paine, 1990). For example, coralline red algae were found to produce metabolites causing maturity suppression in *Laminaria* (Denboh et al., 1997).

Additionally, allelopathic activity has currently been acknowledged as a mechanism in the invasion of a species into a community (Inderjit et al., 2011). Callaway & Aschehoug (2000) formulated the 'novel weapons hypothesis' (NWH). Their proposition projects that exotic invaders will become successful if they bring unique bioactive compounds to their range expansions to which the native, endemic species are not adapted. As the endemic taxa are, consequently, out-competed through these allelopathic interactions, the invasive species is able to establish and proliferate in the new environment, as described by Callaway & Aschehoug (2000). Evidence for this hypothesis has been found in macroalgae as well. For example, settlement of endemic brown, green and red macroalgal propagules, as well as settlement and growth of benthic diatoms, was firmly inhibited on surfaces coated with l,l,3,3-tetrabromo-2-heptanone, an allelochemical isolated from the non-indigenous red algae *Bonnemaisonia hamifera* (Svensson et al., 2013). Moreover, using field and laboratory experiments, *B. hamifera* was found to actively transfer the allelochemical from its own thallus to its native host algae at inhibitory concentrations (Svensson et al., 2013).

Currently, a large percentage of coral reefs globally are on a trajectory of decline, and some of them are already severely degraded (De'ath et al., 2012; Del Monaco et al., 2017; Jackson et al., 2014). One of the symptoms of this downturn is a reduced coverage of reef-building corals and an increased occurrence and abundance of competing macroalgae (e.g., Bruno et al., 2009; Hughes et al., 2010; Rogers and Miller, 2006). Further coral reef decline is expected due to aggravated coral-seaweed competition as macroalgae proliferate and interactions with the remaining corals increase in quantity and quality (Clements et al., 2020; Del Monaco et al., 2017; Hughes et al., 2010). Evidence from both field and laboratory studies indicates that several physical, chemical, and microbial processes can either directly or indirectly moderate coral-seaweed competition, one of them being allelopathy (Clements et al., 2020; Del Monaco et al., 2017). We refer to the review from Chadwick and Morrow (2011) who have described all of these (in)direct mechanisms, both physical and chemical, in detail. Rasher and Hay (2010) observed that 40 to 70 % of the investigated Caribbean and Pacific macroalgae species suppress coral growth due to contact-mediated allelopathy. From literature, it appears that the allelopathic character of macroalgae does not necessarily target a specific life stage of the corals. For instance, the phaeophyte *Lobophora variegata* prevents settlement of larval *Porites astreoides*, while coral larvae of *Acropora palifera* and *Stylophora pistillata* were not found to metamorphose after surface contact with *Lobophora* spp. (Baird and Morse, 2004; Kuffner et al., 2006). On the other hand, Rasher and Hay (2014) described a significant increase in coral bleaching and a reduction of photosynthetic activity of zooxanthellae within 'adult' corals of *Porites cylindrica* after exposure to hydrophobic extracts of the rhodophyte *Galaxaura filamentosa*. Understanding the impacts of coral-macroalgal interactions on the resilience of coral reefs, or the speed of their deterioration, is of crucial significance for conservation efforts of reef ecosystems worldwide.

Potential use of allelopathic agents as natural agrochemicals

In agriculture, allelopathic compounds known to either suppress or eliminate competing plants (crops) have gained specific interest, due to their prospect to be employed as selective natural herbicides (Getachew et al., 2017; Vyvyan, 2002). Nowadays, this interest has partly shifted to marine alternatives, like the development of antifoulants and biocides based on natural products. For example, diterpenes extracted from the brown macroalgae *Dictyota menstrualis* have been found to impede settlement and development of fouling bryozoans (Schmitt et al., 1995). The bioactive compound 1,1,3,3-tetrabromo-2-heptanone, obtained from the rhodophyte *Bonnemaisonia hamifera* through bioassay guided fractionation of hexane surface extracts, was demonstrated to inhibit the growth, at naturally occurring concentrations, of several relevant marine fouling bacteria, that were isolated from macroalgal surfaces (Nylund et al., 2008). Alternatively, several authors have suggested to use the biocidal properties of some seaweed species to steer towards the development and application of environment friendly and cost-effective strategies to control 'harmful algae blooms' (HABs), the excessive growth of deleterious micro- and macroalgae in response to coastal eutrophication (e.g., Nan et al., 2004; Tang and Gobler, 2011; Wang et al., 2006; Table S1).

In contrast to many synthetic compounds, bioactive molecules that are produced by aquatic organisms are both naturally degradable and show high efficiency at low concentrations (Nakai et al., 1996, 2000; Jin & Dong, 2003; Jin et al., 2016). Moreover, indigenous allelopathic species are relatively easy to collect at low cost and they are environmentally benign (Jin et al., 2005; Tang and Gobler, 2011; Wang et al., 2012; Ye et al., 2014). Additionally, many macroalgae taxa have a high nutrient assimilation capacity, hence, potentially simultaneously reducing eutrophication levels of coastal ecosystems significantly, contributing to HAB mitigation (Jin et al., 2005, 2016; Tang and Gobler, 2011; Ye et al., 2014). Both local/regional control authorities as well as the International Maritime Organisation (IMO) legislation are

propulsing towards the design and implementation of natural, non-toxic antifoulants (Bazes et al., 2009; Chambers et al., 2006).

Aquaculture

Allelochemicals of co-occuring seaweed might directly negatively impact the growth, development and physiology of commercially important taxa, such as blue mussel *Mytilus edulis* and oysters *Crassostrea gigas* and *C. virginica* (e.g., Green-Gavrielidis et al., 2018), which is especially relevant in attempts to improve aquaculture yields is to enrich the culture tanks with seaweed. For example, several macroalgae have already been combined into land-based integrated multi-trophic aquaculture (IMTA) systems (e.g., Carl et al., 2014). On the other hand, allelopathic seaweed species might negatively affect and even eliminate competitors, foulers and grazers, co-occurring in the cultivation sites. For instance, *Saccharina latissima* significantly lowered the saxitoxin accumulation in co-cultivated blue mussels by significantly inhibiting the saxitoxin-producing *Alexandrium catenella* (Sylvers and Gobler., 2021).

Biodiscovery of allelochemicals

In screening biologically active compounds derived from marine species, macroalgae have received substantial attention as a natural source of metabolites with promising bioactive properties. These metabolites have been observed to exhibit distinct biological activities, such as cytotoxic, anthelminthic, antibiotic, antineoplastic, antioxidant, antibacterial, antifungal, anti-inflammatory, and cholesterol-lowering activity (Lourenço-Lopes et al., 2020; Kabera et al., 2014). More than 4,000 different metabolites have been extracted from mostly brown and red macroalgae, but from some green seaweed species as well, in the last couple of decades, and have been listed in the DNP, the Dictionary of Natural Products (Lourenço-Lopes et al., 2020). Further characterisation of compounds with allelopathic activities in different species, variation in their bioactivity, and a more profound comprehension of their mode of action, is

recommended to improve the ongoing examination on the potential use of allelochemicals as pharmaceutical agents and bio-herbicides.

4. Conclusion

Macroalgae are a wealthy source of bioactive metabolites with allelopathic potential. To discriminate bioactive metabolites from true allelochemicals it is crucial to verify whether (1) the allelopathic properties are tested using ecologically relevant organisms; (2) the chemicals are encountered by the target organism with reference to the macroalga (on its thallus's exterior or in the surrounding seawater, but not stored inside the seaweed's blade); and (3) the putative allelochemical is bioavailable and inhibits settlement/development/growth at the concentration present at the seaweed surface/released into the water. Even though several macroalgal compounds have been directly or potentially perceived as exhibiting allelochemical properties, there is a clear necessity to improve and strengthen our knowledge on the identity of these compounds, as well as their mode of action. Currently, photosynthesis inhibition in competing photoautotrophs appears to be a frequently employed mechanism of action. Molecular approaches can improve our knowledge on the identity of these compounds, as well as their mode of action. In total, 138 macroalgae species have been described in literature that putatively exhibit allelopathic properties, mostly belonging to a few dominating genera. The allelopathy phenomenon in macroalgae should be further investigated considering several aspects from a scientific and commercial point of view.

Declarations

Ethical Approval

Not applicable

Competing interests

The authors declare no competing interests.

Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

Ilias Semmouri: Data collection, data analysis, Writing - Original Draft, Review & Editing;

Colin Janssen: Supervision, Writing - Review & Editing; **Jana Asselman**: Supervision,

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Availability of data and materials

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