Review

Generation of Reactive Oxygen Species (ROS) by Harmful Algal Bloom (HAB)-Forming Phytoplankton and Their Potential Impact on Surrounding Living Organisms

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Abstract: Most marine phytoplankton with relatively high ROS generation rates are categorized as harmful algal bloom (HAB)-forming species, among which Chattonella genera is the highest ROS-producing phytoplankton. In this review, we examined marine microalgae with ROS-producing activities, with focus on Chattonella genera. Several studies suggest that Chattonella produces superoxide via the activities of an enzyme similar to NADPH oxidase located on glycocalyx, a cell surface structure, while hydrogen peroxide is generated inside the cell by different pathways. Additionally, hydroxyl radical has been detected in Chattonella cell suspension. By the physical stimulation, such as passing through between the gill lamellas of fish, the glycocalyx is easily discharged from the flagellate cells and attached on the gill surface, where ROS are continuously produced, which might cause gill tissue damage and fish death. Comparative studies using several strains of Chattonella showed that ROS production rate and ichthyotoxicity of Chattonella is well correlated. Furthermore, significant levels of ROS have been reported in other raphidophytes and dinoflagellates, such as Cochlodinium polykrikoides and Karenia mikimotoi. Chattonella is the most extensively studied phytoplankton in terms of ROS production and its biological functions. Therefore, this review examined the potential ecophysiological roles of extracellular ROS production by marine microalgae in aquatic environment.

Keywords: reactive oxygen species (ROS); marine microalgae; harmful algae bloom (HAB) species; Chattonella; nitric oxide (NO)

1. Introduction

Over the years, hundreds of marine microalgae, which constitute a significant portion of marine biomass, have been described worldwide. Although several microalgae are known as promising sources of beneficial bioactive compounds, such as anti-cancer and antioxidant agents [1,2], less than 2% of them have been classified as harmful or toxic species [3]. The blooming of dominant algae species at relatively high cell density results in a phenomenon called “red tides”. In the case of harmful or toxic species, it is known as harmful algal blooms (HABs) [4]. The mass growth of specific algal species is easily recognized, often leading to water discoloration [4]. Most HAB species in marine environments are unicellular phytoplankton. HAB is a diverse phenomenon, involving multiple species and classes of microalgae that produce a wide variety of toxins or bioactive compounds, which can negatively affect several aquatic organisms [4–6]. Owing to advances in monitoring, surveillance, and identification technology, there has been an increase in the number of identified harmful and toxic microalgae species. Moreover, there has been an increased awareness of HABs due to an increase in the utilization of coastal areas for aquacultural activities [7,8]. Globalization of logistics seems to contribute to the
distribution of HAB species to new areas through ballast water of ships or contaminated seafoods [9–12]. Recently, there has been an increase in the impact of HABs on fisheries and aquaculture industries. Moreover, the influence of HABs on marine ecosystems and organisms is projected to increase under the present climate change scenario, which could lead to considerable economic and ecological implications.

HABs have considerable implications on commercial and recreational fisheries and coastal tourism, as well as human and wildlife health [3,13–17].

There is emerging evidence that global warming-induced environmental changes may influence the patterns, frequency, distribution, and intensity of HABs in marine, brackish, and freshwater environments [18–21]. Changes in temperature [13], ocean acidification [22], nutrient conditions [23], and the physical structure of the water column [24], have been shown to influence the incidence and global range of HABs.

One of the significant impacts of HABs is the accumulation of algal toxins in shellfish, resulting in food poisoning of humans and animals. Symptoms of shellfish poison in humans include paralytic, diarrhetic, neurotoxic, amnesic, and azaspiracid poisoning [25]. Additionally, toxins and other bioactive compounds produced by algal species during bloom can cause the death of marine organisms. Furthermore, HABs can cause the death of wildlife, including seabirds, whales, dolphins, and other marine animals, through the transfer of toxins via the food web or direct ingestion of toxins [3,16]. Major incidences of HABs-associated mass mortalities and huge economic losses are summarized in Table 1. Although most of the toxins responsible for shellfish poisoning are well defined, the mechanisms of lethal action of harmful species are poorly understood. Overall, raphidophytes and dinoflagellates are the main groups of HAB species. Particularly, species of the *Chattonella* genera (*C. marina*, *C. antiqua*, *C. subsalsa*, *C. minima*, and *C. ovata*) have been reported to cause severe mortality of farmed organisms in mainly temperate and adjacent waters [26–32]. Yellowtail (*Seriola quinqueradiata*), atlantic salmon (*Salmo salar*), northern bluefin tuna (*Thunnus orientalis*), and bluefin tuna (*Thunnus maccaryi*) are especially susceptible to *Chattonella* [32–37]. Mortalities have also been reported in benthic organisms, such as blue crabs, clams, octopus, pen shells, shrimps, and sea cucumber during *Chattonella* blooms [38]. In Japan, *Chattonella* have been reported to cause economic loss of approximately JPY 7.1 billion in the Harima-nada marine region in 1972 [39], and JPY 2.9 billion in 2009 and JPY 5.3 billion in 2010 in the Yatsushiro Sea [40]. Additionally, economic losses to fisheries due to *Chattonella* have been reported in other countries [25,30–32,35,37,38,41–47].

| Species | Main Toxic Factors | Event | References |
|---------|------------------|-------|-----------|
| *Karenia mikimotoi* | Hemolysin; Reactive oxygen species (ROS) | Ichthyotoxic; Toxic to invertebrates; Toxic to zooplankton; Antialgal Molluscicidal | [48–56] |
| *Cochlodinium polykrikoides* | Hemolysin; ROS; Sulfated polysaccharides; Noxiustoxin | Antiviral; Ichthyotoxic; | [57–62] |
| *Eutreptiella gymnastica* | ROS | | [62] |
| *Alexandrium tamarense* | Saxitoxin; Neosaxitoxin; Gonyautoxin; N-sulfocarbomoyl toxins; Tetrodotoxin; Hemolysin; ROS | Neurotoxic; Paralytic shellfish poisoning; Toxic to marine organisms; Toxic to zooplankton; Cytotoxic | [63–83] |
| *Heterosigma akashiwo* (=*Heterosigma carterae*) | Brevetoxin-like toxin; ROS | Ichthyotoxic; Neurotoxic; Toxic to zooplankton | [84–89] |
| *Chattonella marina* | Brevetoxin-like toxin; ROS | Ichthyotoxic; Neurotoxic | [9,88,90–94] |
| *Chattonella ovata* | ROS; Hemolysin; Hemagglutinin | | [95] |
| *Chattonella antiqua* | Brevetoxin-like toxin; ROS | Ichthyotoxic; Neurotoxic | [96–100] |
| *Chattonella subsalsa* | ROS; Hemolysin | | [101] |
| *Platymonas subcordiformis* | | Ichthyotoxic; Toxic to zooplankton | [102] |
Table 1. Cont.

| Species                        | Main Toxic Factors                  | Event                                                                 | References                  |
|--------------------------------|-------------------------------------|----------------------------------------------------------------------|-----------------------------|
| Skeletonema costatum           | ROS                                 | Ichthyotoxic; Toxic to zooplankton; Antibacterial                     | [103–106]                   |
| Olisthodiscus luteus           | ROS                                 | Ichthyotoxic; Antimycotic; Toxic to phytoplankton                     | [88,98,107,108]             |
| Fibrocapsa japonica            | Brevetoxin-like toxin; ROS; Fibrocapsin | Ichthyotoxic; Toxic to marine mammals; Neurotoxic; Toxic to phytoplankton Toxic to mollusks; Antialgal | [88,107,109]                |
| Heterocapsa circularisquama    | ROS; Hemolysin                      | Antiprotozoal; Toxic to zooplankton Ichtivyotoxic; Toxic to mollusks; Antimycotic; Toxic to mice | [110–115]                   |
| Akashiwo sanguineum (=Gymnodinium sanguineum) | ROS                                 | Ichthyotoxic; Toxic to zooplankton Ichtivyotoxic; Toxic to mollusks; Antimycotic; Toxic to mice | [55,98,116,117]             |
| Karlodinium veneficum          | Saxitoxin; Neosaxitoxin; ROS       | Ichthyotoxic; Toxic to zooplankton Neurotoxic; Paralytic shellfish poisoning; Toxic to marine organisms | [55,118,119]                |
| Alexandrium catenella          | Gonyautoxin; N-sulfocarbomoyl toxins; Hemolysin; ROS | Venerupin shellfish poisoning; Toxic to marine organism; Neurotoxic | [73,82,120–126]             |
| Prorocentrum minimum           | Venerupin; Prorocentrin; ß-diketone; ROS | Venerupin shellfish poisoning; Toxic to marine organism; Neurotoxic | [127–139]                   |
| Prymnesium parvum              | Prymnesin 1 and 2; Hemolysin        | Ichthyotoxic; Toxic to tadpoles; Toxic to zooplankton; Cytotoxic     | [140–145]                   |
| Thalassiosira weissflogii      | ROS                                 | Toxic to zooplankton                                                 | [105]                       |
| Thalassiosira pseudonana       | Apo-fucoxanthinoid pigments; ROS   | Toxic to zooplankton                                                 | [146,147]                   |
| Coccolithus sp.                | ROS                                 | Toxic to zooplankton                                                 | [148]                       |
| Pleurochrysis carterae         | ROS                                 | Toxic to zooplankton                                                 | [149]                       |
| Symbiodinium spp.              | ROS                                 | Toxic to zooplankton                                                 | [150]                       |
| Trichodesmium erythraeum       | ROS                                 | Antibacterial; Toxic to marine organisms; Hepatotoxic; Neurotoxic; Ciguatoxin-like | [151–154]                   |
| Prorocentrum micans            | ROS                                 | Shellfish poisoning; Toxic to marine organisms; Antialgal            | [62,155–158]                |

Although several studies have been performed on algal bloom, the exact toxic mechanisms of harmful algae are yet to be elucidated. Previous studies on the ichthyotoxic mechanism of Chattonella have identified several toxic or bioactive compounds involved in fish mortality [39,100,159–165] or the synergistic impact of multiple toxic factors [166]. It has been widely accepted that suffocation is the major cause of fish death by Chattonella [167–170]. Previous findings suggest that the direct target organ of Chattonella is the gill tissue, which can eventually lead to fish death. Matsusato and Kobayashi reported that the dead cells of C. antiqua and cell-free filtrate prepared from the live cell suspension of Chattonella was non-toxic to fish (red sea bream) [171]. Similarly, Ishimatsu et al. [172] also found that lysed cells of C. marina did not kill yellowtail.

Other raphidophytes, such as Heterosigma akashiwo, Olisthodiscus luteus, and Fibrocapsa japonica, also produce extracellular ROS [85,88,160,173,174]. Thus, it seems that ROS production is a common biological feature of raphidophytes. However, extracellular ROS production is not limited to raphidophytes and has been reported in harmful dinoflagellates, including Alexandrium spp. [173,175–177], Margalefidinium polykrikoides (Cochlodinium polykrikoides) [62,178–180], and Karenia mikimotoi [176,181–183].

A recent review showed that phytoplankton are a major producer of ROS, including superoxide and hydrogen peroxide in aquatic environments [184]. Many phytoplankton taxa generate ROS under ordal growth conditions without any stimuli or stress conditions. Although the physiological significance of extracellular ROS production by phytoplankton and their effects on the ecosystem remain unclear, the potential ecological and physiological effects of ROS production include biotoxicity, allelopathy, growth promotion, and iron acquisition. In this review, we described the levels, subcellular mechanism, biological roles,
and toxic potential of ROS production by HABs species, with emphasis on the *Chattonella* genera [184]. Different assay methods have been applied for the detection of ROS produced by marine microalgae, as shown in Table 2.

Additionally, several lines of evidence suggest that *C. marina* can produce nitric oxide (NO) [185], which is involved in various important biological processes in mammals [186], several metabolisms of plants, expression of gene [187], and infectious diseases in plants [188,189]. Moreover, NO and superoxide can form peroxynitrite, a potent oxidant, by reacting to each other. Thus, the mechanism of NO production by *Chattonella* and other species and their biological activities was discussed in the later part of this review.

**Table 2.** List of assay methods used for the detection of reactive oxygen species (ROS) in microalgae.

| ROS                | Studied Algal Species                                                                 | Methods                                           | References                                      |
|--------------------|---------------------------------------------------------------------------------------|---------------------------------------------------|------------------------------------------------|
| Superoxide (O$_2^-$) | *Chattonella* marina, *Chattonella* antiqua, *Karenia* mikimotoi, *Coccolithus* polykrikoides, *Chattonella* ovala, *Olisthodiscus* luteus | 1 MCLA-mediated chemiluminescence assay           | [62,94,159,160,166,181,190–192]                  |
|                    | *Chattonella* marina, *Chattonella* antiqua, *Karenia* mikimotoi                    | 2 L012-mediated chemiluminescence assay           | [164,193–195]                                  |
| Hydroxyl radical (OH$^-$) | *Chattonella* marina                                                                                    | Phenol red assay                                  | [161]                                           |
| Hydrogen peroxide (H$_2$O$_2$) | *Chattonella* marina, *Coccolithus* polykrikoides                                                                 | 3 DMPO-mediated spin trapping method using an ESR | [159,197,198]                                  |
|                    | *Karenia* mikimotoi, *Coccolithus* polykrikoides, *Chattonella* ovala                      | 5 PHPA-mediated fluorescence spectrophotometric assay | [178,181,200]                                  |
| Nitric oxide       | *Chattonella* marina, *Heterosigma* akashiwo, *Chattonella* ovala                             | Phenol red assay                                  | [178,202]                                       |
|                    | *Coccolithus* polykrikoides, *Alexandrium* taylori, *Alexandrium* tamarense, *Nannochloropsis* oculata | 6 DAF-FM DA-mediated fluorometric assay           | [204]                                           |
| Nitric oxide       | *Platymonas subcordiformis, Skeletonema costatum, Gymnodinium sp.*                        | Nitric oxide detection microsensor                | [102]                                           |

1 MCLA, methyl cypridina luciferin analog; 2 L012, 8-amino-5-chloro-7-phenylpyrido [3,4-d]pyridazine-1,4-(2H,3H)-dione; 3 DMPO, 5,5-dimethyl-1-pyrroline N-oxide; 4 red CLA, 2-[4-[4-[5,7-dihydro-2-methyl-3-oxoimidazol[1,2-$\alpha$]-pyrazin-6-yl]benzoxyl]butyramido]ethylaminosulfonohydantoin 101; 5 PHPA, p-hydroxyphenyl acetic acid; 6 DAF-FM DA, 4-Amino-5-methylamino-2,7′-dichlorofluorescein diacetate.

2. Marine Microalgae Species with ROS-Producing Activities

In 1989, Shimada et al. [205] reported the first evidence of ROS production by the raphidophycean flagellate *Chattonella antiqua*. Since then, numerous studies have been conducted
on *Chattonella* spp., including the role of ROS as an ichthyotoxic factor, ROS production mechanism, and the biological roles of ROS in *Chattonella* [28,31,206,207]. Despite extensive studies [86,88,93,100,159,160,166,171,208–212], the exact mechanism through which *Chattonella* cause fish death is still poorly understood. Recently, Shikata et al. [213] examined the ichthyotoxicity of eight strains of *Chattonella* with different backgrounds against different fish species (red sea bream and yellowtail) and found that the generation level of superoxide was most well-correlated with fish-killing activity among several factors examined, which supports the notion that ROS are mainly involved in the *Chattonella*-related fish mortality.

Moreover, few studies have found that other raphidophytes [85,88,173,174] and some dinoflagellates [62,173,175–183] are capable of producing ROS. Furthermore, Marshall et al. [173] examined the superoxide-producing ability of 37 species of microalgae, such as dinoflagellates, raphidophytes, and others, using chemiluminescence analysis, and found that several phytoplankton species are capable of producing superoxide to some extent. Detailed analyses showed a direct correlation between cell size and superoxide production level. Among the species, *Chattonella* produced the highest levels of superoxide per cell, whereas harmless species, such as *Dunaliella*, *Tetraselmis*, *Nannochloropsis*, and *Pavlova*, which are usually used as bivalve feeds, did not produce significant levels of ROS. Furthermore, based on the degree of superoxide production and toxicity, they proposed that phytoplankton species could be classified into four groups. Microalgae producing ROS with exceeded certain threshold value, such as *C. antiqua*, *C. marina*, *C. minima* and *C. ovata*, were categorized as extremely toxic.

According to a review paper on the diversity of phytoplankton in aquatic environments [184], the generation rate of ROS per cell was measured in more than 21 microalgal species; most of them were HAB-forming species [62,85,173,175,182]. The generation rate of one ROS (superoxide; \( \text{O}_2^- \)) has also been quantified in some species of cyanobacteria [148,199,214–216]. Furthermore, HAB species produce higher levels of \( \text{O}_2^- \) than other phytoplankton taxa, including freshwater cyanobacterium *Microcystis aeruginosa* [216], and non-harmful species [196]. These findings indicate that various phytoplankton species are major biological sources of ROS in marine environment, which can cause profound ecological impact on marine environment. Considering the high level of ROS production in marine environments, it is necessary to examine the effects of ROS produced by HAB species, including raphidophytes and dinoflagellates, on marine organisms. Therefore, *Chattonella* spp., other raphidophytes, and dinoflagellates, such as *Cochlodinium polykrikoides* and *Karenia mikimotoi*, were discussed comprehensively in subsequent sections. Table 3 shows details of high ROS-producing marine phytoplankton species.

| Algal Species | ROS | Estimated Production Mechanisms | References |
|--------------|-----|---------------------------------|------------|
| *Karenia mikimotoi* | Superoxide | - | [181,182] |
|          | Hydrogen peroxide | | |
| *Cochlodinium polykrikoides* | Superoxide | - | [159,178,198,217] |
|          | Hydrogen peroxide | SOD catalyzed disproportionation of superoxide (hydrogen peroxide) | |
|          | Hydroxyl radical | • Auto-oxidation of an electron acceptor in photosystem I (superoxide) | |
| *Eutreptiella gymnastica* | Hydrogen peroxide | SOD catalyzed disproportionation of superoxide (hydrogen peroxide) | [62,184] |
| *Prorocentrum micans* | Hydrogen peroxide | SOD catalyzed disproportionation of superoxide (hydrogen peroxide) | [62] |
| *Akashiwo sanguineum* (=*Gymnodinium sanguineum*) | Hydrogen peroxide | SOD catalyzed disproportionation of superoxide (hydrogen peroxide) | [62] |
| *Alexandrium tamarense* | Hydrogen peroxide | SOD catalyzed disproportionation of superoxide (hydrogen peroxide) | [62] |
### Table 3. Cont.

| Algal Species                  | ROS             | Estimated Production Mechanisms                                                                 | References  |
|-------------------------------|-----------------|-------------------------------------------------------------------------------------------------|-------------|
| *Heterosigma akashiwo* (=*H. carterae*) | Superoxide   | • Glycocalyx-mediated ROS generation                                                             | [62,85,162,218] |
|                               | Hydrogen peroxide | • SOD catalyzed disproportionation of superoxide (hydrogen peroxide)                           |             |
|                               | Hydroxyl radical | • NAD(P)H oxidase located in cell surface-bounded glyocalyx (superoxide)                       |             |
|                               | Nitric oxide    | • SOD catalyzed disproportionation of superoxide (hydrogen peroxide)                           |             |
|                               |                 | • NAD(P)H oxidase located in cell surface-bounded glyocalyx (superoxide)                       |             |
|                               |                 | • NAD(P)H oxidase located in cell surface-bounded glyocalyx (superoxide)                       | [162]       |
| *Chattonella marina*          | Superoxide      | • Nitric oxide synthase-like enzyme-mediated mechanism (nitric oxide)                          | [94,162,185,203] |
|                               | Hydrogen peroxide | • Photosynthetic electron transport (superoxide)                                               |             |
|                               | Nitric oxide    | • Photosynthetic electron transport (superoxide)                                               |             |
| *Chattonella ovata*           | Superoxide      | • NAD(P)H oxidase located in cell surface-bounded glyocalyx (superoxide)                       |             |
|                               | Hydrogen peroxide | • SOD catalyzed disproportionation of superoxide (hydrogen peroxide)                           |             |
|                               | Nitric oxide    | • NAD(P)H oxidase located in cell surface-bounded glyocalyx (superoxide)                       | [162]       |
| *Chattonella antiqua*         | Superoxide      | • Photosynthetic electron transport (superoxide)                                               | [193,219]   |
|                               | Hydrogen peroxide | • SOD catalyzed disproportionation of superoxide (hydrogen peroxide)                           |             |
| *Chattonella subsalsa*        | Superoxide      | • Photosynthetic electron transport (superoxide)                                               | [217]       |
| *Platymonas subcordiformis*   | Superoxide      | • Cell surface redox enzyme-mediated mechanism (superoxide)                                    | [102]       |
| *Skeletonema costatum*        | Superoxide      | -                                                                                               | [102]       |
| *Olisthodiscus luteus*        | Superoxide      | • SOD catalyzed disproportionation of superoxide (hydrogen peroxide)                           | [108]       |
| *Fibrocapsa japonica*         | Superoxide      | -                                                                                               | [174]       |
| *Heterocapsa circularisquama*| Hydrogen peroxide | -                                                                                               | [182,184]   |
| *Karenia veneficum*           | Superoxide      | -                                                                                               | [184]       |
| *Alexandrium catenella*       | Superoxide      | -                                                                                               | [184]       |
| *Prorocentrum minimum*        | Hydrogen peroxide | -                                                                                               | [184]       |
| *Prymnesium parvum*           | Superoxide      | -                                                                                               | [184]       |
| *Thalassiosira weissflogii*   | Superoxide      | • NAD(P)H oxidase-related mechanism                                                              | [196,220]   |
| *Thalassiosira pseudonana*    | Hydrogen peroxide | • NAD(P)H oxidase-related mechanism                                                              | [196]       |
| *Thalassiosira oceanica*      | Hydrogen peroxide | • NAD(P)H oxidase-related mechanism                                                              | [221]       |
| *Coscinodiscus sp.*           | Hydrogen peroxide | -                                                                                               | [184]       |
| *Pleurochrysis carterae*      | Hydrogen peroxide | -                                                                                               | [222]       |
| *Symbiodinium spp.*           | Superoxide      | -                                                                                               | [150,223]   |
| *Trichodesmium erythraeum*    | Superoxide      | -                                                                                               | [199]       |

3. Chattonella

Raphidophycean flagellates *Chattonella* spp (*C. marina*, *C. antiqua*, *C. subsalsa*, *C. minima*, and *C. ovata*) are causative species of HAB-associated fish mortality, with serious impact on the aquacultural industry in Japan [31]. Among the genus, *C. marina* and *C. antiqua* are highly toxic species, which are causing enormous negative impact on fish farms in Japan, particularly to yellowtail (*Seriola quinqueradiata*) aquaculture in the last few decades [212]. Additionally, fish mortality due to *Chattonella* spp-induced HABs has occurred in Australia, Netherlands, Brazil, and other parts of the world [28,31,206,207].

Previous studies have proposed several potential toxic factors, such as neurotoxins resembling the brevetoxins produced by *Karenia brevis* (formerly known as *Gymnodinium breve*).
and Ptychodiscus brevis), haemagglutinating agents [86,93,100,210,211], fatty acids [166,212], and mucus substances [171]. Moreover, Shimada et al. [163] reported that C. antiqua has the ability to induce SOD-inhibitable cytochrome c reduction, indicating that live C. antiqua cells can produce superoxide anion. Further studies using several techniques demonstrated that Chattonella spp. generate ROS, such as superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radical (•OH) [88,159,160,208,209]. Since ROS are biologically highly toxic [224,225], Chattonella spp. may exert ichthyotoxicity through ROS at least in part. This hypothesis might be supported by the low toxicity of low superoxide-producing C. marina strains [172,226]. Similarly, Cho et al. [164] reported relatively high ichthyotoxicity of high ROS-producing C. antiqua strains compared with low ROS strain of C. marina. In addition to Chattonella spp., another raphidophycean flagellate, Heterosigma akashiwo, has been reported to show ROS-mediated toxicity against rainbow trout [85]. It is widely accepted that suffocation is the main mechanism of fish death by these flagellates [167–170], with loss of branchial respiratory capacity as an immediate physiological change observed in fish after exposure to Chattonella spp. [31,227]. Exposure of S. quinqueradiata to C. marina at a lethal cell density causes a rapid decrease in the arterial oxygen pressure within less than 30 min [168,169,228], resulting in further physiological responses, such as acidosis [229], ionoregulatory failure [168], increase in circulating catecholamine levels [230], and decrease in cardiac output [228]. Owing to a decrease in arterial oxygen pressure, excess mucus-like substances are secreted, probably by gill tissues, in response to stimulus by C. marina, which cover the gills. Such mucus substance on the gill surface together with glycocalyx, a polysaccharide-containing complex cell surface structure discharged from the flagellate cells, may interfere with O$_2$ uptake from gill lamellas, resulting in asphyxia [169,231,232]. A further in vitro study demonstrated that there was a 26–83% decrease in water flow rate through the excised first gill arch of jack mackerel (Trachurus japonicus) placed in 4000 cells of C. marina/mL for 10 min compared with those placed in culture medium alone, and the gill arch of the flagellate-exposed group was covered with mucus and C. marina cells [233]. In mammalian systems, ROS enhance mucus secretion from various epithelia lining luminal organs, such as the gallbladder of guinea pigs in vitro [234,235], the gastric mucous cells of rats [236], and the tracheal epithelial cells of guinea pigs [237,238]. Considering the similarities between fish and mammals in terms of mucins, mucus cells [239], and secretory mechanisms [240], it could be speculated that ROS produced by C. marina may be involved in the over secretion of mucus on the gill tissue.

Based on previous findings, it seems likely that live cell condition is important for the ichthyotoxicity of Chattonella. Matsusato and Kobayashi [171] reported that neither the dead cells of C. antiqua nor cell-free supernatant of the flagellate culture were toxic to fish. Similarly, Ishimatsu et al. [172] reported that ruptured C. marina showed no toxic effect on yellowtail and found that there is a clear correlation between the cellular O$_2^-$ producing activity and its fish toxicity. The toxin may probably be quite unstable in nature, leading to the disappearance of its activity in ruptured cells and rendering the isolation of the toxin in its active form difficult. Considering these findings, among the toxic factors proposed, ROS is the most provable candidate. Previous studies demonstrated that C. marina suppressed the growth of Vibrio alginolyticus inoculated into plankton culture [160]. The bactericidal activity of C. marina was significantly suppressed by superoxide dismutase (SOD) and catalase, which are antioxidant enzymes with ROS scavenging activity. Additionally, sodium benzoate, a hydroxyl radical scavenger, protected the bacteria from the toxic effect of C. marina. Thus, it was suggested that C. marina can exert negative impact on surrounding bacteria through ROS production. This is an indicative example that ROS-producing marine microalgae, such as Chattonella spp., can cause oxidative stress to surrounding organisms.

3.1. Mechanisms of ROS Production by Chattonella

Various phytoplankton species produce extracellular ROS under normal growth conditions, with Chattonella marina being the highest extracellular ROS producer [184]. ROS pro-
duction by *Chattonella* has been well documented in several independent studies [159–161,163,164,226]. Several techniques have been employed for the detection of each reactive oxygen species; superoxide anions (O$_2^−$) by cytochrome c reduction [160], chemiluminescence analysis [164], and fluorescent microscopy [162,163]; hydrogen peroxide (H$_2$O$_2$) by the phenol red or the scopeoltin assay [161]; and superoxide and hydroxyl radicals (•OH) by electron spin resonance spectroscopy [159,164]. In *Chattonella* cells, ROS production can occur in several major organelles or intracellular compartments, such as chloroplasts, mitochondria, peroxisomes, and cell membrane. The primary oxygen radical-producing step at these sites is the formation of O$_2^−$ via the single electron reduction of O$_2$, and the subsequent enzymatic or non-enzymatic dismutation of superoxide is the most probable mechanism for the production of H$_2$O$_2$. Intracellularly generated H$_2$O$_2$ might easily release extracellularly [94,162]. In contrast, O$_2^−$ is membrane impermeable in nature due to its short life span and limited diffusion distance, indicating that O$_2^−$ hardly crosses cell membranes [241,242]. Thus, the most probable site of O$_2^−$ generation in *Chattonella* may be on the cell surface. To identify the mechanism of O$_2^−$ and H$_2$O$_2$ production, especially focusing on intracellular location of O$_2^−$ and H$_2$O$_2$ production in *C. marina* and *C. ovata*, Kim et al. [162] conducted fluorescence microscopic observation of these flagellate cells using methyl cypridina luciferin analog and 5-(and-6)-carboxy-20,70-dichlorodihydrodihydrofluorescein dictate, acetyl ester, which is a specific fluorescent probe for detecting O$_2^−$ and H$_2$O$_2$, respectively. The fluorescence pictures suggested that superoxide is produced on the cell surface, whereas hydrogen peroxide is produced intracellularly. Furthermore, destruction of the cells by ultrasonic treatment resulted in significant decrease in O$_2^−$ levels, whereas the level of H$_2$O$_2$ detected in the ruptured cells increased as compared to the level of intact cell suspension [161,162]. Thus, the producing mechanisms of O$_2^−$ and H$_2$O$_2$ and their intracellular location seem to be different and independent of each other in the cells.

Generally, it has been considered that hydroxyl radical is the most toxic radical that can destroy proteins, nucleic acids, and other important biomolecules [224,243]. Since hydroxyl radical is detected in the flagellate cells, the ecological impact of these ROS-producing flagellates should be significant. The reaction of superoxide radical and hydrogen peroxide can produce hydroxyl radical. For this reaction, transition metals, such as Fe$^{2+}$ and Cu$^{2+}$, play an important role as reducing agents in the Fenton reaction and the Haber–Weiss cycle [224,243]. Iron is generally required for optimal growth of phytoplankton [244], and the flagellate culture medium contains 0.5 μM EDTA-Fe$^{3+}$ and certain levels of other metal ions. Thus, it is possible that the hydroxyl radical is produced through the Fe-catalyzed Fenton-type Haber–Weiss reaction. This assumption was supported by the fact that hydroxyl radical production in flagellate cell suspension is inhibited by either SOD or catalase [159]. To further evaluate the roles of iron or other metals in hydroxyl radical production, the effect of hypoxanthine/xanthine oxidase addition as a superoxide generation system to flagellate culture medium was examined using electron spin resonance (ESR) analysis, and the results showed that hypoxanthine/xanthine oxidase remarkably increased hydroxyl radical production [159]. Because Fe exists in seawater in the 0.01–1 μM range [245], the formation of hydroxyl radical by *Chattonella* spp. in seawater is feasible.

Tang et al. [246] showed that H$_2$O$_2$ alone was not lethal to fish. It should be noted that *Chattonella* cells produce both superoxide anions and hydrogen peroxide, and the co-occurrence enhances *Chattonella* toxicity to living organisms, which could be due to hydroxyl radical formation [247,248]. Additionally, studies have shown that H$_2$O$_2$ had no effect on mucin release in several cell culture or explant models [235,237,249], whereas hydroxyl radical induced mucus secretion [235]. Since the presence of excessive mucus substance on the gill surface of fish exposed to *Chattonella* is considered as a key factor, hydroxyl radical may play a major role among the ROS in terms of ROS-mediated detrimental effect of *Chattonella* on fish gill.
3.2. NADPH Oxidase as a Superoxide-Anion-Producing Enzyme System

In various biological systems, the generation of extracellular superoxide anion (\(O_2^-\)) is likely regulated by enzyme systems, such as oxidoreductases, utilizing nicotinamide adenine dinucleotide phosphate (NADPH), which acts as a reducing co-factor for the conversion of \(O_2\) to \(O_2^-\). An enzyme NADPH oxidase existing in the plasma membrane of certain white blood cells catalyzes the single-electron reduction of \(O_2\) to \(O_2^-\) [250]. In higher plant cells, there are some NAD(P)H oxidase capable of generating \(O_2^-\) in the plasma membranes, and ROS production in plant cells shows similar characteristic of ROS-generation system so-called oxidative burst in mammalian phagocytic cells [251].

Raphidophytes (C. antiqua, C. marina, H. akashiwo) possess glycocalyx as a cell surface structure [232,252–254], and enzymatic system responsible for \(O_2^-\) generation exists in the glycocalyx, which is easily dissociated from the cells under physical or chemical stimulation [97,191]. To determine the involvement of glycocalyx in ROS generation in C. marina, Kim et al. obtained a supernatant from a C. marina cell suspension by mild agitation, which caused discharge of the glycocalyx without cell destruction [94]. Chemiluminescence assay using \(O_2^-\) specific probe showed that the cell-free supernatant induced SOD-inhibitable strong chemiluminescence in response to exogenous NADPH, whereas the supernatant without NADPH showed only a trace-level response. Additionally, concentration of high molecular weight fraction by ultrafiltration resulted in increased chemiluminescence response, suggesting that certain components with large molecular size in the supernatant are responsible for the reaction. On the other hand, C. marina cell suspension exhibited no response to NADPH. Since NADPH is not membrane permeable, C. marina might not be able to utilize extracellular NADPH. NADH was less effective as compared to NADPH, and NADP\(^+\) and NAD\(^+\) were ineffective. In addition, diphenyleneiodonium, an inhibitor of mammalian NADPH oxidase, prevented NADPH-induced chemiluminescence response in the cell-free supernatant. Probably, C. marina has an enzyme system similar to NADPH oxidase of neutrophil. NADPH oxidase of neutrophil has two subunit proteins in the plasma membrane, gp91phox and p22phox, which form heterodimeric flavocytochrome b558 [250]. To further clarify the \(O_2^-\)-generating enzyme system in C. marina cells, immunoblotting of the cell-free supernatant of C. marina was performed using an antibody raised against neutrophil gp91phox. The result suggested the presence of protein recognized with the antibody in the cell-free supernatant of C. marina. Additionally, indirect immunofluorescence of the flagellate cells using the same antibody indicated that human gp91phox-like protein existed on the surface of C. marina. Furthermore, southern blot using the oligonucleotide probe encoding the C-terminal region of human gp91phox suggested the presence of a gene encoding a protein mimicking gp91phox in C. marina. In addition to several well-described mammalian homologs of gp91phox [255–257], higher plant cells (Arabidopsis thaliana) have slightly larger homologs of gp91phox with 59.8–62.3% sequence similarity to gp91phox [258].

The presence of NADPH oxidase in Chattonella as a source of \(O_2^-\) is further supported by the identification of six putative genes encoding NADPH oxidase (NOX) in C. antiqua [259]. The enzymatic activity of NOX requires NADPH, which is mainly supplied by the oxidative pentose phosphate (OPP) pathway [260,261]. Regarding the regulation mechanism of NOX activity in Chattonella, it has been observed that the production of \(O_2^-\) in C. marina and C. antiqua is inhibited by an inhibitor of photosynthetic electron transport, 3-(3,4-dichlorophenyl)-1,1-dimethylurea [193,262]. These findings suggest that both photosynthesis and OPP pathways are involved in the production of \(O_2^-\) in these flagellate cells. Interestingly, a recent study found that \(O_2^-\) production in C. antiqua increased under nutrient deficiency and suppressed photosynthesis conditions, suggesting that increases in the ratio of NADPH to NADP\(^+\) caused by the OPP pathway might be deeply involved in ROS generation in Chattonella [263].
3.3. Glycocalyx as a Cell Surface Structure with ROS Generation System

Electron and light microscopic observation of *Chattonella antiqua* and *Heterosigma akashiwo* showed that these cells have glycocalyx as the cell surface, which consists of sulfated, non-sulfated polysaccharides, and neutral carbohydrate–protein complex [252,253]. Since raphidophycean flagellates generally do not have a rigid cell wall, glycocalyx may function as a defense or barrier against biological and non-biological invasion. Oda et al. [191] showed that the addition of lectins, such as concanavalin A (Con A), wheat germ agglutinin, and castor bean haemagglutinin, significantly increased in O$_2^-$ generation by *C. marina* and *H. akashiwo*. Since the effects of the lectins were suppressed by specific monosaccharides, the binding of the lectins to the saccharide moieties on the cell surface may have led to increased O$_2^-$ production. Interestingly, high concentration of Con A can induce morphological changes in these flagellate cells. After the addition of Con A, some cells became spherical, distinct from the usual spindle shape, and these changes were frequently accompanied by the discharge of glycocalyx. An analysis using fluorescent-labeled Con A confirmed the binding of Con A to the discharged glycocalyx. These results suggest that the binding of Con A to the glycocalyx is recognized as a stimulus by the flagellate cells, leading to discharge of glycocalyx. Shimada et al. [163] and Tanaka et al. [99] reported that O$_2^-$ was generated in small particles, or in verruciform protrusions located on the cell surface of *C. antiqua*. Additionally, the addition of mucus substances prepared from yellowtail induced the release of these small particles from the flagellate cells. Similarly, Nakamura et al. [218] and Okamoto et al. [254] observed that extracellular addition of mucus substances obtained from yellowtail gill enhanced O$_2^-$ generation by *C. marina*, which was concomitant with the discharge of the glycocalyx. The presence of O$_2^-$ generation system on the glycocalyx, may be supported by the observed suppression of O$_2^-$ generation by *C. marina* and *H. akashiwo* treated with membrane-impermeable protease [191]. In fish mucus, lysozyme, proteases, and lectins and other bioactive molecules have been discovered, as well as mucin, a major mucus compartment [264–266]. Some components in fish mucus that possess lectin activity may act as a stimulus mimicking Con A and induce glycocalyx discharge and activate O$_2^-$ generation.

In addition to lectins or mucus, simple agitation seems to influence the glycocalyx. Matsusato and Kobayashi [171] argued that *Chattonella*-mediated fish mortality could be due to inhibition of respiratory water flow through the gills by mucus substance derived from the flagellate cells. It has been reported that *Chattonella* can secret mucus substances when the cells were passed through a net with 95 µm mesh size. Thus, it could be inferred that mucous substances on the gill surface of fish exposed to *Chattonella* are at least partly derived from *Chattonella* cells. Moreover, indirect immunofluorescence using antiserum raised against crude glycocalyx of *C. marina* suggested the presence of glycocalyx, together with *C. marina* cells on the gill surface of fish exposed to *C. marina* [232]. Ishimatsu et al. [168,229] demonstrated that the earliest physiological and histological changes observed in the yellowtail after *Chattonella* exposure was a rapid drop of arterial oxygen partial pressure and considerable accumulation of mucous substances between the filaments and lamellae of the gill tissues, respectively. Based on the previous studies, it seems obvious that glycocalyx plays a pivotal role in the ichthyotoxic mechanism of *Chattonella* and other raphidophycean flagellates.

4. Raphidophycean Flagellates

*Heterosigma akashiwo, Olisthodiscus luteus*, and *Fibrocapsa japonica* often cause serious mortality of wild and farmed fish [267]. Generation rates of superoxide anion (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) by *C. marina* (two strains), *C. antiqua, H. akashiwo, O. luteus*, and *F. japonica* were estimated by SOD-inhibitable cytochrome c reduction and scopoletin assay, respectively [88]. *Chattonella* showed the highest O$_2^-$ and H$_2$O$_2$ production rates among the raphidophytes tested, based on cell number. This may be due to different cell sizes. *Chattonella* has nearly ten times larger cell size than other raphidophycean flagellates.
Interestingly, an increase in H$_2$O$_2$ levels of disrupted cell suspensions of these raphidophytes even higher than intact flagellate cell suspensions was observed [161]. These findings suggest that these raphidophytes have a certain intracellular compartment where H$_2$O$_2$ might be accumulated at high concentration, from which it is gradually released into the medium during normal growth. The presence of intracellular compartment with high H$_2$O$_2$ concentration may be a common cellular feature of raphidophytes. Regarding the behavior of H$_2$O$_2$, it has been demonstrated that there was a decrease in the concentration of exogenously added H$_2$O$_2$ in *C. marina* cell suspension, with approximately 30 min half-life, whereas H$_2$O$_2$ in the culture medium alone was much more stable [161].

5. Cochlodinium Polykrikoides

*Cochlodinium polykrikoides* is a harmful dinoflagellate with potent fish-killing activity [59,90,268]. Apart from *C. polykrikoides*, *C. fulvescens* [59,269,270] and *Cochlodinium* sp. Type Kasasa [271] have been identified as morphologically similar and toxic species. Cells with 28–35 µm diameter form 4–8 chains, depending on growth conditions. *Cochlodinium* blooms have been reported in Japan, Korea, and other countries [272,273], and cause fish mortality [269,274]. For instance, *C. polykrikoides* bloom caused economic losses of more than USD 100 million to fisheries in Korea [62,275].

Previous studies reported that certain toxic compounds, including neurotoxin, hemolytic toxin, haemagglutininative agent, and paralytic shellfish poisoning (PSP) toxins were found in *C. polykrikoides* [3,58,61,276]. Kim et al. reported that *C. polykrikoides* isolated in Korea generated O$_2^-$ and H$_2$O$_2$ [62] and proposed that *C. polykrikoides* exerts gill tissue damage and fish mortality through ROS production [277]. Contrarily, *C. polykrikoides* isolated in Japan produced O$_2^-$ and H$_2$O$_2$ with much lower levels than those of *Chattonella marina*, and the strains isolated in Japan did not respond to lectins and fish mucus [178]. Further studies showed that only trace levels of ROS were detected in cell suspensions of five clonal strains of *C. polykrikoides* isolated from different localities, and at times in Japan. To evaluate the fish-killing activity of the Japanese strains of *C. polykrikoides* with low ROS generation activity, damselfish (*Chromis caerulea*; average length 3 ± 0.8 cm) were exposed to the flagellates (4 × 10$^3$ cells/mL), and the results showed that damselfish were susceptible to all the strains of *C. polykrikoides* tested, with 100% mortality within 90 min of exposure, whereas no significant protective effects of SOD and catalase were observed in the exposure experiments [194]. The reason for the discrepancy between the Korean and the Japanese strain of *C. polykrikoides* is still unclear. However, since ROS level is dependent on strain, growth conditions, and assay methods [88,173,184], it is possible that the Korean strain may have an extremely more potent ROS generation activity than the Japanese strains. In addition, there is a possibility that certain toxic factors other than ROS might be mainly involved in the ichthyotoxicity of *C. polykrikoides*. Regarding the fish-killing mechanism of *C. polykrikoides*, suffocation caused by huge amount of mucus substances derived from *C. polykrikoides* could be responsible for fish death [61,276]. Exposure experiments using several fish species demonstrated that still-unknown toxic agents together with mucus substances secreted from *C. polykrikoides* may be responsible for fish mortality [269]. Furthermore, *C. polykrikoides* continuously secretes large amounts of mucous substances into the medium as a characteristic feature [178]. Therefore, it could be inferred that the mucus and certain toxins, including ROS, may be involved in the fish-killing mechanism of *C. polykrikoides*. Interestingly, Shin et al. have reported that *C. polykrikoides* induced oxidative damage and DNA degradation in the gill of red seabream after exposure to sub-lethal concentrations of the flagellate [278].

6. Karenia Mikimotoi

The dinoflagellate *Karenia mikimotoi* (*K. mikimotoi*), formerly *Gyrodinium aureolum*, *G. cf. aureolum*, *G. type’65*, *G. nagasakiense*, and *G. mikimotoi*, is highly toxic to both fish and shellfish [279]. *K. mikimotoi* is an unarmed dinoflagellate with average cell size of 23–40 µm in diameter and flattened, with a characteristic swimming [280]. *K. mikimotoi* is a
ectomeric and euryhaline organism, which can survive at temperature range of 4–31 °C and salinity at 9–31 [281,282]. Additionally, K. mikimotoi grows under light intensities (10 to 1200 µmol/m²/s) and can assimilate different chemical forms of nitrogen and phosphorous [53,283].

HABs of K. mikimotoi have occurred in Japanese waters [15,284], the North Atlantic [53,285], and other areas [286,287]. HABs of K. mikimotoi have caused massive mortality of fish [15] and shellfish in Japan [288]. Since the mid-1960s, when K. mikimotoi bloom occurred in Japan [289], mortality of various fish and invertebrate species caused by K. mikimotoi has been reported in Europe, Australia, Japan, South America, and North Africa [3].

Regarding the toxic mechanisms of K. mikimotoi, it has been reported that K. mikimotoi produce several toxic agents, such as low-molecular-weight hemolytic toxins [290–293], cytoxic polyethers [294,295], and ROS [181,296]. Matsuyama reported that G. mikimotoi strongly inhibited the filtration rate of bivalves [297]. Sellem et al. demonstrated that the 18:5n3 fatty acid produced by G. mikimotoi exhibited detrimental effects on sea urchin (Paracentrotus lividus) [298]. Mitchell and Rodger reported that K. mikimotoi bloom was associated with fish and shellfish mortality [299].

In 2012, large-scale HAB of K. mikimotoi (2 × 10⁵–1.18 × 10⁶ cells/mL) caused mass mortality of Japanese pufferfish (Takifugu rubripes) in Japan [300]. Exposure studies confirmed that NGU04, a strain of K. mikimotoi isolated from the HAB area, was toxic to fish during the time (0.3–4 h) in cell-density-dependent manner (5 × 10⁴–1 × 10⁵ cells/mL). Interestingly, NGU04 produced extremely high levels of ROS, which were nearly equal to the levels of C. marina measured at the same time [183]. ROS generation by C. marina increases in response to extracellular stimuli, such as lectins [191,218]. After binding to the cell surface carbohydrate moieties, lectins induce various cellular signaling pathways, leading to the enhancement of ROS generation in leukocytes [301,302]. C. marina might possess such pathways regulating ROS generation in response to lectin stimuli, as found in leukocytes. NGU04 also increased ROS levels in the presence of three lectins, which had different saccharide specificity. Additionally, the lectin response profile of NGU04 differed from that of C. marina. This may reflect differences in cell surface structures between raphidophycean flagellate C. marina and dinoflagellate K. marina, especially the lectin binding sites [183].

Regarding O₂⁻ and H₂O₂ generation mechanisms, Hymenomonas carterae, a marine phytoplankton, produced extracellular H₂O₂ without utilizing O₂⁻ [222]. Similarly, fluorescence microscopic observation of the NGU04, using ROS-specific fluorescence probes, indicated that O₂⁻ and H₂O₂ are produced in different intracellular compartments [183].

The zooplankton Brachionus plicatilis (B. plicatilis) is highly susceptible to K. mikimotoi [303], with NGU04 exhibiting the most lethal effect against B. plicatilis among the K. mikimotoi strains tested, whereas C. marina had no significant effect on the zooplankton under the same experimental conditions [183]. Since the toxic potential of K. mikimotoi on marine organisms, including shellfish, is well correlated with its toxicity against rotifer [303,304], the response of rotifer to the algae could be a reliable assay for evaluating K. mikimotoi toxicity. Moreover, a recent review [305] indicated that rotifer could be useful for marine ecotoxicology studies. Thus, the results suggest that NGU04 can exert potential toxicity on shellfish, as well as on fish. As supporting evidence for this, NGU04 demonstrated that it was lethal against juvenile abalone (Nordotis gigantea) in laboratory experiments [306]. Furthermore, NGU04 exerted hemolytic activities against rabbit and fish erythrocytes, and the activities were much stronger than other strains of K. mikimotoi with different backgrounds [306]. Since antioxidant enzymes, such as SOD and catalase, had no effect on rotifer toxicity of NGU04, it could be inferred that ROS might not be the major toxic factor of the strain, at least against rotifer [183]. Therefore, it is possible that K. mikimotoi exerts its toxic effect against rotifer and shellfish mainly through its hemolytic activity. This notion is supported by the non-toxic effect of C. marina, with no hemolytic activity against rotifer; however, Heterocapsa circularisquama with potent hemolytic activity can kill rotifer and shellfish, but not fish.
Overall, it could be concluded that *K. mikimotoi*, especially at high cell density blooms, can negatively affect several surrounding organisms, not only through ROS-mediated oxidative stress, but via its hemolytic activity.

7. Nitric Oxide (NO) Production in Marine Microalgae

Over the years, studies have shown that *C. marina* generates nitric oxide (NO) under ordinal growth conditions [185]. As a first experiment, chemiluminescence (CL) reaction between NO and luminol–$\text{H}_2\text{O}_2$ was employed to detect NO in *C. marina* [307]. When $\text{H}_2\text{O}_2$ and luminol were added to *C. marina* simultaneously, increased CL response was detected, and it was significantly suppressed by 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (carboxy-PTIO), a specific NO scavenger [308]. Detailed kinetic analyses indicated that NO production by *C. marina* was cell-density-dependent, and the level of NO for $10^4$ cells/mL was estimated to be nearly 10 $\mu$M. Griess reaction also confirmed the NO production by *C. marina* [309]. Furthermore, NO was detected in *C. marina* cell suspension using NO-reactive fluorescent probe diaminofluorescein-FM diacetate (DAF-FM DA) technique; however, NO fluorescence was completely inhibited by carboxy-PTIO [310]. Moreover, fluorescence microscopic observation suggested that NO was generated intracellularly in *C. marina*, and bright fluorescence of *C. marina* was inhibited by carboxy-PTIO. In addition, a comparative study showed that significantly higher fluorescence was detected in raphidophytes *Chattonella ovata* and *Heterosigma akashiwo*, whereas only trace levels of fluorescence were observed in dinophytes *Alexandrium tamarense*, *A. taylori*, *Cochlodinium polykrikoides*, *Gymnodinium impudicum*, and eustigmatophycean *Nannochloropsis oculata*. These results suggest that relatively high level of NO production may be a common specific feature of the raphidophycean flagellates [204]. To analyze the mechanism of NO production in *C. marina*, the effect of an inhibitor of NO synthase (NOS), NG-Nitro-L-arginine methyl ester (L-NAME) was examined. L-NAME is known to block NO production in mouse macrophage cell line RAW264.7 cells [311]. Similarly, L-NAME inhibited NO production by *C. marina*. In contrast, the addition of L-arginine, a substrate for NOS, resulted in an increase in NO level, with results obtained by luminol–$\text{H}_2\text{O}_2$ assay.

NO, a gaseous free radical initially described as an endothelium-derived relaxing factor [312], is involved in various biological processes in mammals [186]. NO plays numerous roles not only in animals but also in plants. NO is membrane permeable and is involved in many important processes in plants [187–189]. Apart from higher plants, green algae and cyanobacteria also produce NO [313–315]. Similarly to *C. marina*, some species of marine microalgae also generate NO under certain conditions [102,316,317]. The results obtained by three independent assay methods demonstrated that *C. marina* is capable of producing NO without specific stimuli or stress conditions. In mammalian systems, NO is mainly produced from L-arginine by NOS, which yields L-citrulline and NO [318]. In plants, nitrate reductase (NR) has been demonstrated as a major NO generation system [319]. Moreover, NO is also produced by non-enzymatic nitrite reduction at acidic conditions [320]. In contrast to *C. marina*, it has been reported that unicellular alga *Chlamydomonas reinhardtii* produces NO through NR activity [315], and not through the activity of NOS-like enzyme. As observed in *C. reinhardtii*, NO production by *Chlorella sorokiniana* occurred in darkness and required nitrite [321]. Furthermore, it has been suggested that the NR-mediated NO production activity of these microalgae was linked with photosynthetic electron transport system because illumination of the algae cells suppresses NO production; however, the suppressive effect can be reversed by 3,4-dichlorophenyl-1,1-dimethyleurea, a photosynthesis inhibitor [315]. Thus, it is likely that these unicellular algae may have a common NO production mechanism that can be affected by various growth conditions. Regarding *C. marina*, continuous NO production was observed under normal growth conditions (under illumination), and exogenous nitrite had no effect, suggesting that NR may not be involved in NO generation. Similarly to *C. marina*, some evidence suggests the presence of NOS-like activities in photosynthetic organisms [316,322]. Furthermore, a pathogen-induced NO-synthesizing enzyme has been purified from tobacco leaves [323].
Such pathogen-induced NO production in plants may play an important role in defense mechanism against pathogens through hypersensitive response [189]. NO has been shown to be an important regulator of mucus secretion in the stomach [324–326], and NO donors induced mucus secretion from isolated gastric mucus cells [325]. Therefore, it is conceivable that NO alone or in combination with ROS may induce over-secretion of mucus substances on the gill surface of fish exposed to C. marina cells, leading to the blockage of respiratory water flow. Shimada et al. [327] observed the presence of highly concentrated NOx in the cortex of Chattonella antiqua, and they proposed that NOx may induce mucus discharge from gill surface when C. antiqua cells pass between gill lamellae. NO [328] and its oxidized stable product, nitrite [329], are also known to oxidize hemoglobin to methemoglobin that cannot transport oxygen, leading to tissue hypoxia [330]. Regarding histological findings in the gills of fish exposed to C. marina, it has been reported that C. marina induced mucus secretion and altered gill lamella integrity in goldlined seabream after exposure, but no significant increase in methemoglobin was observed in the fish, even after developing symptoms [331].

Apart from the possible factor involved in fish-killing mechanism of C. marina, NO concentration of marine environments is $10^4$ times higher than atmospheric NO level due to extensive photolysis of nitrate and nitrification processes [332], and some species of microalgae release NO during the natural growth process [102,317]. Overall, these findings suggest that NO could be a stressor of marine organisms in multiple ways.

For a quick overview of this review, the most harmful and notable HAB-forming species, together with some topics specific to the species, are summarized in Table 4. C. marina, C. antiqua, C. polykrikoides, and Karenia mikimotoi are well-recognized ROS-producing harmful algae, and ROS seem to play pivotal roles in the ichthyotoxic mechanisms, while hemolysin might be responsible for shellfish toxicity.

| Species                               | Main Toxic Factors Detected                                      | Susceptible Organisms | Topics                                                                 |
|---------------------------------------|------------------------------------------------------------------|-----------------------|-----------------------------------------------------------------------|
| Chattonella marina/antiqua (Raphidophyte) | 1 ROS (superoxide, hydrogen peroxide, and hydroxyl radical)   | Fish                  | 1 NADPH oxidase is proposed as a mechanism of ROS production, which might be located on glycocalyx, a cell surface structure [94,162,193,219]. |
|                                       | 2 Nitric oxide (NO)                                              |                       | 2 The highest ROS generation rate among the species tested so far [184]. |
| Cochlodinium polykrikoides (Dinoflagellate) | 1 Hemolysin                                                      | Fish                  | 1 Secretion of huge amount of highly viscous mucus-like substances [61,276]. |
|                                       | 2 ROS (superoxide and hydrogen peroxide)                        | Shellfish             |                                                                       |
| Karenia mikimotoi (Dinoflagellate)    | 1 Hemolysin                                                      | Fish                  | 1 Extremely toxic to both fish and shellfish, and HABs due to this dinoflagellate are often associated with mass mortality of both fish and shellfish [279,299]. |
|                                       | 2 ROS (superoxide and hydrogen peroxide)                        | Shellfish             |                                                                       |

8. Conclusions

HABs are a serious threat to marine resources and fisheries. Anthropogenic changes, including global warming, can further increase the distribution of HABs and the appearance of new HAB species. However, studies are yet to comprehensively elucidate the toxic mechanism of HAB species. In this review, we focused on raphidophytes (Chattonella marina, C. antiqua, and Heterosigma akashiwo) and dinoflagellates (Karenia mikimotoi and Cochlodinium polykrikoides), which are the major groups of HAB species. Since HABs of these species frequently cause mortality of wild and farmed marine organisms, with huge economic losses (Table 4), it is necessary to have a comprehensive understanding of the HAB species, their toxic mechanisms, and their blooming period or conditions, which may help in minimizing their impact.
The findings of this review showed that most of the harmful algae, especially the ichthyotoxic species described above, produced relatively higher levels of ROS, with *Chattonella* having the highest production rate. Although extensive studies are required to fully understand the biological significances of ROS production by HAB species and their impact on surrounding ecosystems and organisms, it is probable that ROS play pivotal roles in the fish-killing activities of HAB species, such as *Chattonella*, which is supported by the findings of previous studies. ROS production alone may not sufficiently explain the ichthyotoxic mechanism of the HAB species and could be attributed to synergistic effects of multiple factors. Therefore, additional studies are needed for a comprehensive elucidation of the synergistic effects of multiple factors in the fish-killing activities of flagellate cells. For instance, the biochemical and cellular structural characteristics of *Chattonella* and the physiological vulnerability of gill tissue of susceptible fish species to the flagellates should be examined. Although studies have shown that *Chattonella* extracts possess several bioactive compounds with hemolytic [3,333] and antioxidant [334–337] activities, their biological significance is still an open question. Further efforts can help elucidate the exact roles of ROS and other bioactive molecules and the detailed processes leading to eventual fish death. Overall, current findings on *Chattonella* are summarized in a schematic diagram (Figure 1).

![Production of reactive oxygen species (ROS) and other bioactive molecules in *Chattonella*.](image)

**Figure 1.** Production of reactive oxygen species (ROS) and other bioactive molecules in *Chattonella*.

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References

1. Lauritano, C.; Andersen, J.H.; Hansen, E.; Albrigtsgen, M.; Escalera, L.; Esposito, F.; Helland, K.; Hanssen, K.O.; Romano, G.; Janora, A. Bioactivity screening of microalgae for antioxidant, anti-inflammatory, anticancer, anti-diabetes, and antibacterial activities. Front. Mar. Sci. 2016, 3, 68. [CrossRef]

2. Andretta, E.; Costa, C.; Longobardi, C.; Damiano, S.; Giordano, A.; Pagnini, F.; Montagnaro, S.; Quintiliani, M.; Lauritano, C.; Ciarcia, R. Potential approaches versus approved or developing chronic myeloid leukemia therapy. Front. Oncol. 2021, 11, 801779. [CrossRef]

3. Landsberg, J.H. The effects of harmful algal blooms on aquatic organisms. Rev. Fish. Sci. 2002, 10, 113–390. [CrossRef]

4. Zohdi, E.; Abbaspour, M. Harmful algal blooms (red tide): A review of causes, impacts and approaches to monitoring and prediction. Int. J. Environ. Sci. Technol. 2019, 16, 1789–1806. [CrossRef]

5. Cho, K.; Heo, J.; Han, H.D.; Hong, C.Y.; Kim, D.; Han, J.W.; Back, K. Industrial applications of Dinoflagellate phycotoxins based on their modes of action: A review. Toxins 2020, 12, 805. [CrossRef] [PubMed]

6. Grattan, L.M.; Holobaugh, S.; Morris, J.G., Jr. Harmful algal blooms and public health. Harmful Algae 2016, 57, 2–8. [CrossRef] [PubMed]

7. Rensel, J.E.; Whyte, J.N.C. Finfish mariculture and harmful algal blooms. Man. Harmful Mar. Microalgae. Monogr. Oceanogr. Methodol. 2003, 11, 693–722.

8. Smayda, T.J.; Villareal, T.A. The 1985 ‘brown-tide’ and the open phytoplankton niche in Narragansett Bay during summer. In Novel Phytoplankton Blooms; Springer: Berlin/Heidelberg, Germany, 1989; pp. 159–187.

9. Hallegraeff, G.M. Transport of toxic dinoflagellates via ships ballast water: Bioeconomic risk assessment and efficacy of possible ballast water management strategies. Mar. Ecol. Prog. Ser. 1998, 168, 297–309. [CrossRef]

10. Honjo, T. Potential transfer of Heterocapsa circularisquama with pearl oyster consignments. Harmful Algae 1998, 224–226.

11. Hégaret, H.; Wikfors, G.H. Time-dependent changes in hemocytes of eastern oysters, Crassostrea virginica, and northern bay scallops, Argopecten irradians irradians, exposed to a cultured strain of Proocentrum minimum. Harmful Algae 2005, 4, 187–199. [CrossRef]

12. Matsuyama, Y.; Nishitani, G.; Nagai, S. Direct detection of harmful algae from the oyster spat and live fish transport trailers. In Proceedings of the XIII International Conference on Harmful Algae, Hong Kong, China, 6 November 2008; pp. 185–189.

13. Hallegraeff, G.M. A review of harmful algal blooms and their apparent global increase. Phycologia 1993, 32, 79–99. [CrossRef]

14. Anderson, D.M. Red tides. Sci. Am. 1994, 271, 62–68. [CrossRef]

15. Honjo, T. The biology and prediction of representative red tides associated with fish kills in Japan. Rev. Fish. Sci. 1994, 2, 225–253. [CrossRef]

16. Shumway, S.E. A review of the effects of algal blooms on shellfish and aquaculture. J. World Aquacult. Soc. 1990, 21, 65–104. [CrossRef]

17. Matsuyama, Y.; Shumway, S.E. Impacts of harmful algal blooms on shellfisheries aquaculture. In New Technologies in Aquaculture; Burnell, G., Allan, G., Eds.; Woodhead Publishing: Sawston, UK, 2009; pp. 580–609.

18. Berdalet, E.; Fleming, I.E.; Gowen, R.; Davidson, K.; Hess, P.; Backer, L.C.; Moore, S.K.; Hoagland, P.; Enevoldsen, H. Marine harmful algal blooms, human health and wellbeing: Challenges and opportunities in the 21st century. J. Mar. Biol. Assoc. UK 2016, 96, 61–91. [CrossRef] [PubMed]

19. Gobler, C.J.; Doherty, O.M.; Hattenrath-Lehmann, T.K.; Griffith, A.W.; Kang, Y.; Litaker, R.W. Ocean warming since 1982 has expanded the niche of toxic algal blooms in the North Atlantic and North Pacific oceans. Proc. Natl. Acad. Sci. USA 2017, 114, 4975–4980. [CrossRef]

20. McCabe, R.M.; Hickey, B.M.; Kudela, R.M.; LeFebvre, K.A.; Adams, N.G.; Bill, B.D.; Gulland, F.M.D.; Thomson, R.E.; Cochlan, W.P.; Trainer, V.L. An unprecedented coastwide toxic algal bloom linked to anomalous ocean conditions. Geophys. Res. Lett. 2016, 43, 10–366. [CrossRef] [PubMed]

21. Paerl, H.W. Impacts of Climate Change on Cyanobacteria in Aquatic Environments; Caister Academic Press: Poole, UK, 2016; pp. 5–22.

22. Granelli, E.; Haraldsson, C. Can increased leaching of trace metals from acidified areas influence phytoplankton growth in coastal waters? Ambio 1993, 22, 308–311.

23. Anderson, D.M.; Glibert, P.M.; Burkholler, J.M. Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. Estuaries 2002, 25, 704–726. [CrossRef]

24. León-Muñoz, J.; Urbina, M.A.; Garreau, R.; Iriarte, J.L. Hydroclimatic conditions trigger record harmful algal bloom in western Patagonia (summer 2016). Sci. Rep. 2018, 8, 1330. [CrossRef]

25. Anderson, D.M.; Fensin, E.; Gobler, C.J.; Hoeglund, A.E.; Hubbard, K.A.; Kulis, D.M.; Landsberg, J.H.; LeFebvre, K.A.; Provoost, P.; Richlen, M.L.; et al. Marine harmful algal blooms (HABs) in the United States: History, current status and future trends. Harmful Algae 2021, 102, 101975. [CrossRef] [PubMed]
26. Ono, C.; Takano, H. *Chattonella antiqua* (Hada) comb. nov., and its occurrence on the Japanese coast. *Bull. Tokai Reg. Fish. Res. Lab.* 1980, 102, 93–100.

27. Simaková, N.V.; Orlova, T.; Selina, M.S. Red tide caused by raphidophytes *Chattonella sp.* Amurskii Bay, the Sea of Japan. *Russ. J. Mar. Biol.* 1990, 16, 77–79.

28. Munday, B.L.; Hallegraeff, G.M. Mass mortality of captive southern bluefin tuna (*Thunnus maccocygi* in April/May 1996 in Boston Bay, South Australia: A complex diagnostic problem. *Fish Pathol.* 1998, 33, 343–350. [CrossRef]

29. Smyrda, T.J. *Ecophysiology and bloom dynamics of Heterosigma akashiwo* (Raphidophyceae), In *Physiological ecology of harmful algal blooms*; Anderson, D.M., Cembella, A.D., Hallegraeff, G.M., Eds.; Springer: Berlin, Germany, 1998; pp. 113–131.

30. Edvardsen, B.; Imai, I. The ecology of harmful flagellates within Prymnesiothphycaceae and Raphidophyceae. In *Ecology of Harmful Algae*; Springer: Berlin/Heidelberg, Germany, 2006; pp. 67–79.

31. Imai, I.; Yamaguchi, M. Life cycle, physiology, ecology and red tide occurrences of the fish-killing raphidophyte *Chattonella*. *Harmful Algae* 2012, 14, 46–70. [CrossRef]

32. García-Mendoza, E.; Caceras-Martinez, J.; Rivas, D.; Fimbres-Martinez, M.; Sanchez-Bravo, Y.; Vásquez-Yemans, R.; Medina-Elizalde, J. Mass mortality of cultivated northern bluefin tuna *Thunnus thynnus orientalis* associated with *Chattonella species* in Baja California, Mexico. *Front. Mar. Sci.* 2018, 5, 454. [CrossRef]

33. Okaichi, T. *Akishio no Kagaku*; Koseisha-Koseikaku: Tokyo, Japan, 1987; p. 294.

34. Okaichi, T. *Red-Tide Phenomena*; Terra Scientific Publishing Company: Tokyo, Japan, 2003; pp. 7–60.

35. Hallegraeff, G.M.; Munday, B.L.; Baden, D.G.; Whitney, P.L. *Chattonella marina* raphidophyte bloom associated with mortality of cultured bluefin tuna *Thunnus maccocygi* in southern Australia. In *Harmful Algae*; Reguera, B., Blanco, J., Ferandiz, M.L., Wyatt, T., Eds.; Xunta de Galicia and Compostela, Spain, 1998; pp. 93–96.

36. Hallegraeff, G.M.; Anderson, D.M.; Belin, C.; Bottein, M.Y.D.; Bresnan, E.; Chinain, M.; Enevoldsen, H.; Iwataki, M.; Ikawa, M.; Karlson, B.; McKenzie, C.H.; et al. Perceived global increase in algal blooms is attributable to intensified monitoring and emerging bloom impacts. *Commun. Earth Environ.* 2021, 2, 1–10. [CrossRef]

37. McKenzie, C.H.; Bates, S.S.; Martin, J.L.; Haigh, N.; Howland, K.L.; Lewis, N.I.; Locke, A.; Peña, A.; Poulin, M.; Rockon, A.; et al. Three decades of Canadian marine harmful algal events: Phytoplankton and phycotoxins of concern to human and ecosystem health. *Harmful Algae* 2021, 102, 101852. [CrossRef]

38. Barraza-Guardado, R.; Cortés-Altamirano, R.; Sierra-Beltrán, A. Marine die-offs from *Chattonella marina* and *Ch. cf. ovata* in Kun Kaak Bay, Sonora in the Gulf of California. *Harmful Algae News* 2004, 16, 7–8.

39. Okaichi, T. Red tide in the Seto Inland Sea. In *Sustainable Development in the Seto Inland Sea, Japan—From the Viewpoint of Fisheries*; Okaichi, T., Yanagi, T., Eds.; Terra Scientific Publishing Company: Tokyo, Japan, 1997; pp. 251–304.

40. Ono, C.; Takano, H. Algal blooms in the Seto inland sea, Japan—from the viewpoint of fisheries. *Fish Pathol.* 2011, 46, 70. [CrossRef]

41. Onitsuka, G.; Aoki, K.; Shimizu, M.; Matsuuya, Y.; Kimoto, K.; Matsuoh, H.; Kitadai, Y.; Nishi, H.; Tahara, Y.; Sakurada, K. Short-term dynamics of a *Chattonella antiqua* bloom in the Yatsushiro Sea, Japan, in summer 2010: Characteristics of its appearance in the southern area. *Bull. Jpn. Soc. Fish. Oceanogr.* 2011, 75, 143–153.

42. Domingos, P.; Menezes, M. Taxonomic remarks on planktonic phytoflagellates in a hypertrophic tropical lagoon (Brazil). In *Phytoplankton and Trophic Gradients*; Springer: Dordrecht, The Netherlands, 1998; pp. 297–313.

43. Hallegraeff, G.M.; Schweibold, L.; Jaffreziec, E.; Rhodes, L.; MacKenzie, L.; Hay, B.; Farrell, H. Overview of Australian and New Zealand harmful algal species occurrences and their societal impacts in the period 1985 to 2018, including a compilation of historic records. *Harmful Algae* 2021, 102, 101848. [CrossRef]

44. Marshall, J.A. Comparative Ecophysiology, Chemotaxonomy and lichthotoxicity of *Chattonella marina* (Raphidophyceae) from Australia and Japan. Ph.D. Thesis, University of Tasmania, Tasmania, Australia, 2003.

45. Scat, D.; Satta, C.T.; Casabianca, S.; Penna, A.; Padedda, B.M.; Sechi, N.; Lugli, A. Identification of *Chattonella* (Raphidophyceae) species in long-term phytoplankton samples from Santa Giusta Lagoon, Italy. *Sci. Mar.* 2016, 80, 17–25.

46. Sakamoto, S.; Lim, W.A.; Lu, D.; Dai, X.; Orlova, T.; Iwataki, M. Harmful algal blooms and associated fisheries damage in East Asia: Current status and trends in China, Japan, Korea and Russia. *Harmful Algae* 2021, 102, 101787. [CrossRef]

47. Sunesen, I.; Méndez, S.M.; Mancera-Pineda, J.E.; Bottein, M.Y.D.; Enevoldsen, H. The Latin America and Caribbean HAB status report based on OBIS and HAEDAT maps and databases. *Harmful Algae* 2021, 102, 101920. [CrossRef]

48. Yigituez, A.T.; Lim, P.T.; Leaw, C.P.; Jipanin, S.J.; Iwataki, M.; Benico, G.; Azanza, R.V. Over 30 years of HABs in the Philippines and Malaysia: What have we learned? *Harmful Algae* 2021, 102, 101776. [CrossRef] [PubMed]

49. Abe, T.; Hirayama, K. Lethal effect of *Gymnodinium cf. plicatilis*. *Bull. Fac. Fish. 1979, 46, 1–6.

50. Li, X.; Yan, T.; Yu, R.; Zhou, M. A review of *Karenia mikimotoi*: Bloom events, physiology, toxicity and toxic mechanism. *Harmful Algae* 2019, 90, 101702. [CrossRef]

51. Gentien, P.; Arzul, G. Exotoxin production by *Gymnodinium cf. aureolum* (Dinophyceae). *J. Mar. Biol. Assoc. UK* 1990, 70, 571–581. [CrossRef]

52. Yasumoto, T. Marine microorganisms toxins—An overview. In *Toxic Marine Phytoplankton*; Granéli, E., Sundstrom, B., Edler, L., Anderson, D.M., Eds.; Elsevier: New York, NY, USA, 1990; pp. 3–8.

53. Arzul, G.; Bodennec, G.; Erard, E.; Gentien, P. Fish kills and *Gymnodinium cf. nagasakiiense in Corsica* (France). *Harmful Algae News* 1994, 8, 7.

54. Gentien, P. Bloom dynamics and ecophysiology of the *Gymnodinium mikimotoi* species complex. In *Physiological Ecology of Harmful Algal Blooms*; Anderson, D.M., Cembella, A.D., Hallegraeff, G.M., Eds.; Springer: Berlin/Heidelberg, Germany, 1998; pp. 155–173.
54. Parrish, C.C.; Bodnenec, G.; Gentien, P. Haemolytic glycolipids from Gymnodinium species. Phytochemistry 1998, 47, 783–787. [CrossRef]
55. Daugbjerg, N.; Hansen, G.; Larsen, J.; Moestrup, Ø. Phylogeny of some of the major genera of dinoflagellates based on ultrastructure and partial LSU rDNA sequence data, including the erection of three new genera of unarmoured dinoflagellates. Phycologia 2000, 39, 302–317. [CrossRef]
56. Hansen, G.; Daugbjerg, N.; Henriksen, P. Comparative study of Gymnodinium mikimotoi and Gymnodinium aureolum comb. nov. (=Gyrodinium aureolum) based on morphology, pigment composition, and molecular data. J. Phycol. 2000, 36, 394–410. [CrossRef]
57. Ho, M.-S.; Zubkoff, P.L. The effects of a Cochlodinium heterolobatum bloom on the survival and calcium uptake by larvae of the American oyster, Crassostrea virginica. In Toxin Dinoflagellate Blooms; Taylor, D.L., Seliger, H.H., Eds.; Elsevier: New York, NY, USA, 1979; pp. 409–412.
58. Onoue, Y.; Nozawa, K. Zinc-bound PSP toxins separated from Chlorella in red tide. In Mycotoxins and Phycotoxins ’88; Natori, S., Hashimoto, K., Ueno, Y., Eds.; Elsevier: Amsterdam, The Netherlands, 1989; pp. 359–366.
59. Yuki, K.; Yoshimatsu, S. Two Fish-Killing Species of Cochlodinium from Harima Nada, Seto Inland Sea, Japan; Elsevier: New York, NY, USA, 1989; pp. 451–454.
60. Hasui, M.; Matsuda, M.; Okutani, K.; Shigeta, S. In vitro antiviral activities of sulfated polysaccharides from a marine microalga (Cochlodinium polykrrikoide) against human immunodeficiency virus and other enveloped viruses. Int. J. Biol. Macromol. 1995, 17, 293–297. [CrossRef]
61. Lee, J.S. Bioactive components from red tide plankton, Cochlodinium polykrrikoide. J. Korean Fish. Soc. 1996, 29, 165–173.
62. Kim, C.S.; Lee, S.G.; Kim, H.G.; Jung, J. Reactive oxygen species as causative agents in the ichthyotoxicity of the red tide dinoflagellate Cochlodinium polykrrikoide. J. Plankton Res. 1999, 21, 2105–2115. [CrossRef]
63. Needler, A.B. Paralytic shellfish poisoning and Gonyaulax tamarensis. J. Fish. Res. Board Can. 1949, 7, 490–504. [CrossRef]
64. Prakash, A. Source of paralytic shellfish toxin in the Bay of Fundy. J. Fish. Res. Board Can. 1963, 20, 983–996. [CrossRef]
65. Prakash, A. Growth and toxicity of a marine dinoflagellate, Gonyaulax tamarensis. J. Fish. Res. Board Can. 1967, 24, 1589–1606. [CrossRef]
66. White, A.W.; Maranda, L. Paralytic shellfish toxins in the dinoflagellate Gonyaulax excavata and in shellfish. J. Fish. Res. Board Can. 1978, 35, 397–402. [CrossRef]
67. Oshima, Y.; Yasumoto, T. Analysis of toxins in cultured Gonyaulax excavata. In Toxic Dinoflagellate Blooms; Taylor, D.L., Seliger, H.H., Eds.; Elsevier: New York, NY, USA, 1979; pp. 377–380.
68. Betz, J.M.; Blogoslawski, W.J. Toxicity of Gonyaulax tamarensis var. excavata cells to the brine shrimp Artemia salina L. J. Pharm. Sci. 1981, 71, 463–465.
69. White, A.W. Sensitivity of marine fishes to toxins from the red-tide dinoflagellate Gonyaulax excavata and implications for fish kills. Mar. Biol. 1981, 65, 255–260. [CrossRef]
70. Anderson, D.M.; Kulis, D.M.; Orphanos, J.A.; Ceurvels, A.R. Distribution of the toxic dinoflagellate Gonyaulax tamarensis in the southern New England region. Estuar. Coast. Shelf Sci. 1982, 14, 447–458. [CrossRef]
71. Schantz, E.J. Historical perspective on paralytic shellfish poisons. In Seafood Toxins; Ragelis, E.P., Ed.; American Chemical Society: Washington, DC, USA, 1984; pp. 99–111.
72. Maranda, L.; Anderson, D.M.; Shimizu, Y. Comparison between populations of Gonyaulax tamarensis of eastern North America waters. Estuar. Coast. Shelf Sci. 1985, 21, 401–410. [CrossRef]
73. Ogata, T.; Kodama, M. Ichthyotoxicity found in cultured media of Protogonyaulax spp. Mar. Biol. 1986, 92, 31–34. [CrossRef]
74. Ogata, T.; Kodama, M.; Ishimaru, T. Toxin production in the dinoflagellate Protogonyaulax tamarensis. Toxicon 1987, 25, 923–928. [CrossRef]
75. Cembella, A.D.; Sullivan, J.J.; Boyer, G.L.; Taylor, F.J.R.; Andersen, R.J. Variation in paralytic shellfish toxin composition within the Protogonyaulax tamarensis/catenella species complex; red tide dinoflagellates. Biochem. Syst. Ecol. 1987, 15, 171–186. [CrossRef]
76. Cembella, A.D.; Therriault, J.-C.; Beland, P. Toxicity of cultured isolates and natural populations of Protogonyaulax tamarensis from the St. Lawrence Estuary. J. Shellfish Res. 1988, 7, 611–621.
77. Lassus, P.; Frémy, J.-M.; Ledoux, M.; Bardouil, M.; Bohec, M. Patterns of experimental contamination by Protogonyaulax tamarensis in some French commercial shellfish. Toxicon 1989, 27, 1313–1321. [CrossRef]
78. Kodama, M.; Ogata, T.; Sato, S.; Nakamoto, S. Possible association of marine bacteria with paralytic shellfish toxicity in bivalves. Mar. Ecol. Prog. Ser. 1990, 61, 203–208. [CrossRef]
79. Kodama, M.; Sato, S.; Ogata, T. Alexandrium tamarense as a source of tetrodotoxin in the scallop Patinopecten yessoensis. In Toxic Phytoplankton Blooms in the Sea; Smayda, T.J., Shimizu, Y., Eds.; Elsevier: Amsterdam, The Netherlands, 1993; pp. 401–406.
80. Kodama, M.; Sato, S.; Nakamoto, S.; Ogata, T. Occurrence of tetrodotoxin in Alexandrium tamarense: A causative dinoflagellate of paralytic shellfish poisoning. Toxicon 1996, 34, 1101–1105. [CrossRef]
81. Lee, J.S.; Jeon, J.-K.; Han, M.-S.; Oshima, Y.; Yasumoto, T. Paralytic shellfish toxins in the mussel Mytilus edulis and dinoflagellate Alexandrium tamarense from Jinhae Bay, Korea. Bull. Korean Fish. Soc. 1992, 25, 144–150. [CrossRef]
82. Kim, C.-H.; Sako, Y.; Ishida, Y. Comparison of toxin composition between populations of Alexandrium spp. from geographically distinct areas. Nippon Suisan Gakkaishi 1993, 59, 641–646. [CrossRef]
83. Perovic, S.; Trettel, L.; Brummer, F.; Wetzler, C.; Brenner, J.; Donner, G.; Schroder, H.C.; Muller, W.E. Dinoflagellates from marine algal blooms produce neurotoxic compounds: Effects on free calcium levels in neuronal cells and synaptosomes. *Environ. Toxicol. Pharmacol.* 2000, 8, 83–94. [CrossRef]

84. Chang, E.H.; Anderson, C.; Boustead, N.C. First record of a *Heterosigma* (Raphidophyceae) bloom with associated mortality of cage-reared salmon in Big Glory Bay, New Zealand. *N. Z. J. Mar. Fresh.* 1990, 24, 461–469. [CrossRef]

85. Yang, C.Z.; Albright, L.J.; Yousif, A.N. Oxygen-radical-mediated effects of the toxic phytoplankter *Heterosigma carterae* on juvenile rainbow trout *Oncorhynchus mykiss*. *Dis. Aquat. Org.* 1995, 23, 101–108. [CrossRef]

86. Khan, S.; Arakawa, O.; Onoue, Y. Neurotoxins in a toxic red tide of *Heterosigma akashiwo* (Raphidophyceae) in Kagoshima Bay. *Japan. Aquac. Res.* 1997, 28, 9–14. [CrossRef]

87. Lush, G.J.; Hallegraeff, G.M. High toxicity of the red tide dinoflagellate *Alexandrium minutum* to the brine shrimp *Artemia salina*. In *Harmful and Tox Algal Blooms*; Yasumoto, T., Oshima, Y., Fukuyo, Y., Eds.; Intergovernmental Oceanographic Commission of UNESCO: Paris, France, 1996; pp. 389–392.

88. Oda, T.S.; Nakamura, A.; Okamoto, T.; Komatsu, N.; Oda, T.; Ishimatsu, A.; Muramatsu, T. Generation of reactive oxygen species by the red tide phytoplankton *Chattonella antiqua*. *Harmful Algae* 2000, 22, 1961–1975. [CrossRef]

89. Twiner, M.J.; Trick, C.G. Possible physiological mechanisms for production of hydrogen peroxide by the ichthyotoxin flagellate *Heterosigma akashiwo*. *J. Plankton Res.* 2000, 22, 137–141. [CrossRef]

90. Onoue, Y.; Nozawa, K. Separation of toxins from harmful red tides occurring along the coast of Kagoshima Prefecture. In *Red Tides, Biology, Environmental Science and Toxicology*; Okaichi, T., Anderson, D.M., Nemoto, T., Eds.; Elsevier: New York, NY, USA, 1989; pp. 371–374.

91. Onoue, Y.; Haq, M.S.; Nozawa, K. Separation of neurotoxins from *Chattonella marina*. *Nippon Suisan Gakkaishi* 1990, 56, 695. [CrossRef]

92. Ahmed, M.S.; Arakawa, O.; Onoue, Y. Toxicity of cultured *Chattonella marina*. In *Harmful Marine Algal Blooms*; Lasuss, P., Arzul, G., Erard-Le-Denn, E., Gentien, P., Marcaillou-Le-Baut, C., Eds.; Lavoisier: New York, NY, USA, 1995; pp. 499–504.

93. Khan, D.; Ahmed, M.S.; Arakawa, O.; Onoue, Y. Properties of neurotoxins separated from a harmful red tide organism *Chattonella marina*. *Isr. J. Aquac.* 1995, 47, 137–141.

94. Kim, D.; Nakamura, A.; Komatsu, N.; Oda, T.; Iida, T.; Ishimatsu, A.; Muramatsu, T. Mechanisms of superoxide anion generation in the toxic red tide phytoplankton *Chattonella marina*: Possible involvement of NAD(P)H oxidase. *Biochim. Biophys. Acta* 2000, 1520, 220–227. [CrossRef]

95. Hiroishi, S.; Okada, H.; Imai, I.; Yoshida, T. High toxicity of the novel bloom-forming species *Chattonella ovata* (Raphidophyceae) to cultured fish. *Harmful Algic* 2005, 4, 783–787. [CrossRef]

96. Shimada, M.; Murakami, T.H.; Imahayashi, T.; Ozaki, H.S.; Toyoshima, T.; Okaichi, T. Effects of sea bloom, *Chattonella antiqua*, on gill primary lamellae of the young yellowtail, *Seriola quinquemaculata*. *Acta Histochem. Cytotochem.* 1983, 16, 232–244. [CrossRef]

97. Shimada, M.; Akagi, N.; Nakai, Y.; Goto, H.; Watanabe, M.; Watanabe, H.; Nakanishi, M.; Yoshimatsu, S.; Ono, C. Free radical production by the red tide alga, *Chattonella antiqua*. *Histochem. J.* 1991, 23, 361–365. [CrossRef] [PubMed]

98. Nagai, H.; Satake, M.; Morata, M.; Yasumoto, T. Screening of marine phytoplankton for antifungal substances. In *Toxic Marine Phytoplankton*; Granelli, E., Sundstrom, B., Edler, L., Anderson, D.M., Eds.; Academic Press: New York, NY, USA, 1990; pp. 385–390.

99. Tanaka, K.; Muto, Y.; Shimada, M. Generation of superoxide anion radicals by the marine phytoplankton organism, *Chattonella antiqua*. *J. Plankton Res.* 1994, 16, 161–169. [CrossRef]

100. Khan, S.; Arakawa, P.O.; Onoue, Y. A toxicological study of the marine phytoflagellate, *Chattonella antiqua* (Raphidophyceae). *Phycologia* 1996, 35, 239–244. [CrossRef]

101. Viana, T.V.; Fistarol, G.O.; Amario, M.; Menezes, R.B.; Carneiro, B.L.; Cavalcante, H.; Dias, M.P.; Silva, J.; Tenenbaum, D.R.; et al. Massive blooms of *Chattonella subsalsa* Biecheler (Raphidophyceae) in a hypernutrophic, tropical estuary—Guanabara Bay, Brazil. *Front. Mar. Sci.* 2019, 6, 85. [CrossRef]

102. Zhang, Z.B.; Liu, C.Y.; Wu, Z.Z.; Xing, L.; Li, P.F. Detection of nitric oxide in culture media and studies on nitric oxide formation by marine microalgae. *Med. Sci. Monit.* 2006, 12, BR75–BR85. [PubMed]

103. Ianora, A.; Poulet, S.A.; Miralto, A. A comparative study of the inhibitory effect of diatoms on the reproductive biology of the copepod *Acartia clausi*. *Mar. Biol.* 1995, 121, 533–539. [CrossRef]

104. Kent, M.L.; Whyte, J.N.C.; LaTrace, C. Gill lesions and mortality in seawater pen-reared Atlantic salmon *Salmo salar* associated with a dense bloom of *Skeletomena costatum* and *Thalassiosira* species. *Dis. Aquat. Org.* 1995, 22, 77–81. [CrossRef]

105. Ban, S.; Burns, C.; Castel, J.; Chaudron, Y.; Christou, E.; Escribano, R.; Fonda Umani, S.; Gasparini, S.; Guerrero Ruiz, F.; Hoffmeyer, M.; et al. The paradox of diatom–copepod interactions. *Mar. Ecol. Prog. Ser.* 1997, 157, 287–293. [CrossRef]

106. Naviner, M.; Berge, J.-P.; Durand, P.; Le Brum, H. Antibacterial activity of the marine diatom *Seriola quinqueradiata* (Raphidophyceae). *Fish Pathol.* 2000, 35, 1658–1662. [CrossRef] [PubMed]

107. Uye, S.; Takamatsu, K. Feeding interactions between planktonic copepods and red-tide flagellates from Japanese coastal waters. *Mar. Ecol. Prog. Ser.* 1996, 59, 97–107. [CrossRef]

108. Kim, D.; Nakamura, A.; Okamoto, T.; Komatsu, N.; Oda, T.; Ishimatsu, A.; Muramatsu, T. Toxic potential of the raphidophyte *Olisthodiscus luteus*: Mediation by reactive oxygen species. *J. Plankton Res.* 1999, 21, 1017–1027. [CrossRef]

109. Khan, S.; Arakawa, P.O.; Onoue, Y. Neurotoxin production by a chloromonad, *Fibrocapsa japonica* (Raphidophyceae). *J. World Aquac. Soc.* 1996, 27, 254–263. [CrossRef]
110. Matsuyama, Y.; Nagai, K.; Mizuguchi, T.; Fujiwara, M.; Ishimura, M.; Yamaguchi, M.; Uchida, T.; Honjo, T. Ecological features and mass mortality of pearl oysters during the red tide of Heterocapsa sp. in Ago Bay in 1992. *Nippon Suisan Gakkaishi* 1995, 61, 35–41. [CrossRef]

111. Uchida, T.; Yamaguchi, M.; Matsuyama, Y.; Honjo, T. The red-tide dinoflagellate Heterocapsa sp. kills Gyrodinium instrictum by cell contact. *Mar. Ecol. Prog. Ser.* 1995, 118, 301–303. [CrossRef]

112. Kamiyama, T. Growth and grazing responses of tintinnid ciliates feeding on the toxic dinoflagellate Heterocapsa circularisquama. *Mar. Biol.* 1997, 128, 509–515. [CrossRef]

113. Kamiyama, T.; Arima, S. Lethal effect of the dinoflagellate Heterocapsa circularisquama upon the tintinnid ciliate Favela tairaikaensis. *Mar. Ecol. Prog. Ser.* 1997, 160, 27–33. [CrossRef]

114. Kim, D.; Sato, Y.; Oda, T.; Muramatsu, T.; Matsuyama, Y.; Honjo, T. Specific toxic effect of dinoflagellate Heterocapsa circularisquama on the rotifer Brachionus plicatilis. *Biosci. Biotechnol. Biochem.* 2000, 64, 2719–2722. [CrossRef] [PubMed]

115. Oda, T.S.; Sato, Y.; Kim, D.; Muramatsu, T.; Matsuyama, Y.; Honjo, T. Hemolytic activity of Heterocapsa circularisquama (Dinophyceae) and its possible involvement in shellfish toxicity. *J. Physcol.* 2001, 37, 509–516. [CrossRef]

116. Marshall, S.M.; Orr, A.P. On the biology of *Calanus finmarchicus*. VIII. Food uptake, assimilation and excretion in adult and stage V *Calanus*. *J. Mar. Biol. Assoc. UK* 1953, 34, 495–529. [CrossRef]

117. Tindall, D.R.; Dickey, R.W.; Carlson, R.D.; Morey-Gaines, G. Ciguatoxigenic dinoflagellates from the Caribbean Sea. In *Toxins*; Ragelis, E.P., Ed.; American Chemical Society Symposium Series: Washington, DC, USA, 1984; pp. 225–240.

118. Proctor, N.H.; Chan, S.L.; Trevor, A.J. Production of saxitoxin by cultures of *Prorocentrum*. *J. Shellfish Res.* 1991, 10, 575–587. [CrossRef]

119. Boyer, G.L.; Sullivan, J.J.; Andersen, R.J.; Harrison, P.J.; Taylor, F.J.R. Toxin production in three isolates of *Prorocentrum* sp. in Australian waters. *J. Mar. Biol. Assoc. UK* 1953, 34, 169–189. [CrossRef]

120. Schantz, E.J.; Lynch, J.M.; Vayvada, G.; Matsutomo, K.; Rapoport, H. The purification and characterization of the poison produced by *Gonyaulax catenella* in axenic culture. *Biochemistry* 1966, 5, 1191–1195. [CrossRef]

121. Onoue, P.; Noguchi, T.; Hashimoto, K. Studies on paralytic shellfish poison from the oyster cultured in Senzaki Bay, Yamaguchi Prefecture. *Nippon Suisan Gakkaishi* 1980, 46, 1031–1034. [CrossRef]

122. Marshall, S.M.; Orr, A.P. On the biology of *Calanus finmarchicus*. VIII. Food uptake, assimilation and excretion in adult and stage V *Calanus*. *J. Mar. Biol. Assoc. UK* 1953, 34, 495–529. [CrossRef]

123. Cardwell, R.D.; Olsen, S.; Carr, M.I.; Sanborn, E.W. Causes of Oyster Mortality in South Puget Sound; Washington Department of Fisheries, Salmon Research and Development: Brinnan: Washington, DC, USA, 1979.

124. Nakazima, M. Studies on the source of shellfish poison in Lake Hamana. I. Relation of the abundance of a species of the dinoflagellate *Protogonyaulax* from Senzaki Bay, Yamaguchi Prefecture. *Nippon Suisan Gakkaishi* 1965, 31, 198–203. [CrossRef]

125. Nakazima, M. Studies on the occurrence of toxic dinoflagellates. *Mar. Ecol. Prog. Ser.* 1983, 19, 301–308. [PubMed]

126. Hallegraeff, G.M.; Bolch, C.J.; Blackburn, S.I.; Oshima, Y. Species of the toxigenic dinoflagellate genus *Gymnodinium*. *Nippon Suisan Gakkaishi* 1997, 63, 219–226. [CrossRef]

127. Nakazima, M. Studies on the source of shellfish poison in Lake Hamana. II. Shellfish toxicity during the “Red-Tide”. *Nippon Suisan Gakkaishi* 1965, 31, 204–207. [CrossRef]

128. Nakazima, M. Studies on the source of shellfish poison in Lake Hamana. III. Poisonous effects of shellfishes feeding on *Prorocentrum* sp. *Nippon Suisan Gakkaishi* 1965, 31, 281–285. [CrossRef]

129. Nakazima, M. Studies on the source of shellfish poison in Lake Hamana. IV. Identification and collection of the noxious dinoflagellate. *Nippon Suisan Gakkaishi* 1968, 34, 130–131. [CrossRef]

130. Okai, T.; Imatomi, Y. Toxicity of *Prorocentrum minimum* var. *mariae-lebouriae* assumed to be a causative agent of short-necked clam poisoning. In *Toxic Dinoflagellate Blooms*; Taylor, D.L., Seliger, H.H., Eds.; Elsevier: New York, NY, USA, 1979; pp. 385–388.

131. Andersen, R.J.; LeBlanc, J.J.; Sum, F.W. 1–(2,6,6-trimethyl-4-hydroxy-cyclo-hexenyl)-1,3-butanedione, an extracellular metabolite from *Prorocentrum*. *J. Org. Chem.* 1980, 45, 1169–1170. [CrossRef]

132. Tricker, C.G.; Andersen, R.J.; Gillam, A.; Harrison, J.P. Prorocentrum: An extracellular siderophore produced by the marine dinoflagellate *Prorocentrum*. *Science* 1983, 219, 306–308. [CrossRef] [PubMed]

133. Okaichi, T.; Imatomi, Y. Toxicity of *Prorocentrum minimum* var. *mariae-lebouriae* assumed to be a causative agent of short-necked clam poisoning. In *Toxic Dinoflagellate Blooms*; Taylor, D.L., Seliger, H.H., Eds.; Elsevier: New York, NY, USA, 1979; pp. 385–388.

134. Andersen, R.J.; LeBlanc, J.J.; Sum, F.W. 1–(2,6,6-trimethyl-4-hydroxy-cyclo-hexenyl)-1,3-butanedione, an extracellular metabolite from *Prorocentrum*. *J. Org. Chem.* 1980, 45, 1169–1170. [CrossRef]

135. Luckenbach, M.W.; Sellner, K.G.; Shumway, S.E.; Greene, K. Effects of two bloom-forming dinoflagellates, *Prorocentrum minimum* and *Gyrodinium uncatenatum*, on the growth and survival of the eastern oyster (*Gmelin, 1791*). *J. Shellfish Res.* 1993, 12, 411–415.

136. Sikorski, G.H.; Smolowitz, R.M. Detrimental effects of a *Prorocentrum isolate* upon hard clams and bay scallops in laboratory feeding studies. In *Toxic Phytoplankton Blooms in the Sea*; Smayda, T.J., Shimizu, Y., Eds.; Elsevier: Amsterdam, The Netherlands, 1993; pp. 447–452.

137. Sikorski, G.H.; Smolowitz, R.M. Experimental and histological studies of four life-history stages of the eastern oyster, *Crassostrea virginica* exposed to a cultured strain of the dinoflagellate *Prorocentrum minimum*. *Biol. Bull.* 1995, 188, 313–328. [CrossRef] [PubMed]

138. Sikorski, G.H.; Smolowitz, R.M.; Smith, B.C. Effects of a *Prorocentrum isolate* upon the oyster, *Crassostrea virginica*: A study of three life-history stages. *J. Shellfish Res.* 1993, 12, 114–115.

139. Grzybik, D.; Demard, A.; Berland, B.; Pouchus, Y.F. Evidence of a new toxin in the red-tide dinoflagellate *Prorocentrum minimum*. *J. Plankton Res.* 1997, 19, 1111–1114. [CrossRef]
Antioxidants 2022, 11, 206

139. Denardou-Quenezhervea, A.; Grzebyk, D.; Pouchus, Y.-F.; Sauviat, M.P.; Alliot, E.; Biard, J.-F.; Berland, B.; Verbit, J.-F. Toxicity of French strains of the dinoflagellate Procerocentrum minimum experimental and natural contaminations of mussels. Toxicon 1999, 37, 1711–1719. [CrossRef]

140. Shilo, M. The toxic principles of Prymnesium parvum. In The Water Environment: Algal Toxins and Health; Carmichael, W.W., Ed.; Plenum Press: New York, NY, USA, 1981; pp. 37–47.

141. Kozakai, H.; Oshima, Y.; Yasumoto, T. Isolation and structural elucidation of hemolysin from the phytoflagellate Prymnesium parvum. Agric. Biol. Chem. 1982, 46, 233–236. [CrossRef]

142. Igarashi, T.; Satake, M.; Yasumoto, T. Prymnesin-2: A potent ichthyotoxic and hemolytic glycoside isolated from the red tide alga Prymnesium parvum. J. Am. Chem. Soc. 1992, 114, 479–480. [CrossRef]

143. Igarashi, T.; Satake, M.; Yasumoto, T. Prymnesin-2: A potent ichthyotoxic and hemolytic glycoside isolated from the red tide alga Prymnesium parvum. J. Am. Chem. Soc. 1992, 114, 479–480. [CrossRef]

144. Meldahl, A.-S.; Edvardsen, B.; Fonnum, F. Toxicity of four potentially ichthyotoxic marine phytoflagellates determined by four different test methods. J. Toxicol. Environ. Health 1994, 42, 289–301. [CrossRef]

145. Moestrup, Ø. Economic aspects: ‘Blooms’, nuisance species, and toxins. In Marine Algal Blooms; Smayda, T.J., Shimizu, Y., Eds.; Elsevier: Amsterdam, The Netherlands, 1993; pp. 223–228.

146. Endean, R.; Griffith, J.K.; Robbins, J.J.; Monks, S.A. Multiple toxins in a specimen of the narrow-barred Spanish mackerel, Trachurus symmetricus. Toxicon 1993, 31, 195–204. [CrossRef]

147. Hansel, C.M.; Buchwald, C.; Diaz, J.M.; Oleynik, S.I.; Van Mooy, B.A.S.; Polivyo, D. Dynamics of extracellular superoxide production by Trichodesmium colonies from the Sargasso Sea. Limnol. Oceanogr. 2016, 61, 1188–1200. [CrossRef]

148. Hansel, C.M.; Buchwald, C.; Diaz, J.M.; Oleynik, S.I.; Van Mooy, B.A.S.; Polivyo, D. Dynamics of extracellular superoxide production by Trichodesmium colonies from the Sargasso Sea. Limnol. Oceanogr. 2016, 61, 1188–1200. [CrossRef]

149. Hansel, C.M.; Buchwald, C.; Diaz, J.M.; Oleynik, S.I.; Van Mooy, B.A.S.; Polivyo, D. Dynamics of extracellular superoxide production by Trichodesmium colonies from the Sargasso Sea. Limnol. Oceanogr. 2016, 61, 1188–1200. [CrossRef]

150. Houdan, A.; Bonnard, A.; Fresnel, J.; Fouchard, S.; Billard, C.; Probert, I. Toxicity of coastal coccolithophores (Prymnesiophyceae, Haptophyta). J. Plankton Res. 2004, 26, 875–883. [CrossRef]

151. Houdan, A.; Bonnard, A.; Fresnel, J.; Fouchard, S.; Billard, C.; Probert, I. Toxicity of coastal coccolithophores (Prymnesiophyceae, Haptophyta). J. Plankton Res. 2004, 26, 875–883. [CrossRef]

152. Ramamurthy, V.D. Antibacterial activity of the marine blue-green alga Trichodesmium erythraeum. Curr. Sci. 1967, 19, 524–525.

153. Ramamurthy, V.D. Antibacterial activity of the marine blue-green alga Trichodesmium erythraeum in the gastro-intestinal contents of the sea gull Larus brunicephalus. Mar. Biol. 1967, 6, 74–76. [CrossRef]

154. Endean, R.; Griffith, J.K.; Robbins, J.J.; Monks, S.A. Multiple toxins in a specimen of the narrow-barred Spanish mackerel, Trachurus symmetricus. Toxicon 1993, 31, 195–204. [CrossRef]

155. Endean, R.; Griffith, J.K.; Robbins, J.J.; Monks, S.A. Multiple toxins in a specimen of the narrow-barred Spanish mackerel, Trachurus symmetricus. Toxicon 1993, 31, 195–204. [CrossRef]

156. Endean, R.; Griffith, J.K.; Robbins, J.J.; Monks, S.A. Multiple toxins in a specimen of the narrow-barred Spanish mackerel, Trachurus symmetricus. Toxicon 1993, 31, 195–204. [CrossRef]

157. Pinto, J.S.; Silva, E.S. The toxicity of coastal coccolithophores (Prymnesiophyceae, Haptophyta). J. Plankton Res. 2004, 26, 875–883. [CrossRef]

158. Pinto, J.S.; Silva, E.S. The toxicity of coastal coccolithophores (Prymnesiophyceae, Haptophyta). J. Plankton Res. 2004, 26, 875–883. [CrossRef]

159. Pinto, J.S.; Silva, E.S. The toxicity of coastal coccolithophores (Prymnesiophyceae, Haptophyta). J. Plankton Res. 2004, 26, 875–883. [CrossRef]

160. Cho, K.; Sakamoto, J.; Noda, T.; Nishiguchi, T.; Ueno, M.; Yamasaki, Y.; Yagi, M.; Kim, D.; Oda, T. Comparative studies on the fish-killing activities of Chattonella marina isolated in 1985 and Chattonella antiqua isolated in 2010, and their possible toxic factors. Biosci. Biotechnol. Biochem. 2016, 80, 811–817. [CrossRef]

161. Cho, K.; Sakamoto, J.; Noda, T.; Nishiguchi, T.; Ueno, M.; Yamasaki, Y.; Yagi, M.; Kim, D.; Oda, T. Comparative studies on the fish-killing activities of Chattonella marina isolated in 1985 and Chattonella antiqua isolated in 2010, and their possible toxic factors. Biosci. Biotechnol. Biochem. 2016, 80, 811–817. [CrossRef]

162. Cho, K.; Sakamoto, J.; Noda, T.; Nishiguchi, T.; Ueno, M.; Yamasaki, Y.; Yagi, M.; Kim, D.; Oda, T. Comparative studies on the fish-killing activities of Chattonella marina isolated in 1985 and Chattonella antiqua isolated in 2010, and their possible toxic factors. Biosci. Biotechnol. Biochem. 2016, 80, 811–817. [CrossRef]

163. Cho, K.; Sakamoto, J.; Noda, T.; Nishiguchi, T.; Ueno, M.; Yamasaki, Y.; Yagi, M.; Kim, D.; Oda, T. Comparative studies on the fish-killing activities of Chattonella antiqua isolated in 2010, and their possible toxic factors. Biosci. Biotechnol. Biochem. 2016, 80, 811–817. [CrossRef]
166. Marshall, J.A.; Nichols, P.D.; Hamilton, B.; Lewis, R.J.; Hallegraeff, G.M. Ichthyotoxicity of *Chattonella marina* (Raphidophyceae) to damselfish (*Acanthochromis polyacanthus*): The synergistic role of reactive oxygen species and free fatty acids. *Harmful Algae* 2003, 2, 273–281. [CrossRef]

167. Endo, M.; Foscarini, R.; Kuroki, A. Electrocardiogram of a marine fish, *Pagrus major*, exposed to red tide plankton, *Chattonella marina*. *Mar. Biol.* 1998, 97, 477–481. [CrossRef]

168. Ishimatsu, A.; Maruta, H.; Tsuchiyama, T.; Ozaki, M. Respiratory, ionoregulatory and cardiovascular responses of the yellowtail *Seriola quinquergiata* to exposure to the red tide plankton *Chattonella*. *Nippon Suisan Gakkaishi* 1990, 56, 189–199. [CrossRef]

169. Ishimatsu, A.; Sameshima, M.; Tamura, A.; Oda, T. Histological analysis of the mechanisms of *Chattonella*-induced hypoxemia in yellowtail. *Fish. Sci.* 1996, 62, 50–58. [CrossRef]

170. Ishimatsu, A.; Maruta, H.; Oda, T.; Ozaki, M. A comparison of physiological responses in yellowtail to fatal environmental hypoxia and exposure to *Chattonella marina*. *Fish. Sci.* 1997, 63, 557–562. [CrossRef]

171. Matsusato, T.; Kobayashi, H. Studies on death of fish caused by red tide. *Bull. Nansei Reg. Fish. Res. Lab.*

172. Ishimatsu, A.; Sameshima, M.; Tamura, A.; Oda, T. Histological analysis of the mechanisms of *Chattonella*-induced hypoxemia in yellowtail. *Fish. Sci.* 1996, 62, 50–58. [CrossRef]

173. Marshall, J.A.; de Salas, M.; Oda, T.; Hallegraeff, G. Superproduction by marine microalgae: I. Survey of 37 species from 6 classes. *Mar. Biol.* 2005, 147, 533–540. [CrossRef]

174. Portune, K.J.; Cary, S.C.; Warner, M.E. Antioxidant enzyme response and reactive oxygen species production in marine raphidophytes. *C. Pharm.* 2013, 46, 1161–1171. [CrossRef]

175. Mooney, B.D.; Dorantes-Aranda, J.J.; Place, A.R.; Hallegraeff, G.M. Ichthyotoxicity of gymnodinoid diatoms and dinoflagellates: PUFA and superoxide effects in sheephead minnow larvae and rainbow trout gill cells. *Mar. Ecol. Prog. Ser.* 2011, 426, 213–224. [CrossRef]

176. Dorantes-Aranda, J.J.; Seger, A.; Mardones, J.I.; Nichols, P.D.; Hallegraeff, G.M. Progress in understanding algal bloom-mediated fish kills: The role of superoxide radicals, phycotoxins and fatty acids. *PLoS ONE* 2015, 10, e0133549. [CrossRef]

177. Mardones, J.I.; Dorantes-Aranda, J.J.; Nichols, P.D.; Hallegraeff, G.M. Fish gill damage by the dinoflagellate *Alexandrium catenella* from Chilean fjords: Synergistic action of ROS and PUFA. *Harmful Algae* 2015, 49, 40–49. [CrossRef]

178. Kim, D.; Oda, T.; Muramatsu, T.; Kim, D.; Matsuyama, Y.; Honjo, T. Possible factors responsible for the toxicity of *Cochlodinium polykrikoides*, a red tide phytoplankton. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* 2002, 132, 415–423. [CrossRef]

179. Tang, Y.Z.; Gobler, C.J. *Cochlodinium polykrikoides* blooms and clonal isolates from the northwest Atlantic coast cause rapid mortality in larvae of multiple bivalve species. *Mar. Biol.* 2009, 156, 2601–2611. [CrossRef]

180. Griffith, A.W.; Gobler, C.J. Temperature controls the toxicity of the ichthyotoxic dinoflagellate *Cochlodinium polykrikoides*. *Mar. Ecol. Prog. Ser.* 2016, 545, 63–76. [CrossRef]

181. Yamasaki, Y.; Kim, D.I.; Matsuyma, Y.; Oda, T.; Honjo, T. Production of superoxide anion and hydrogen peroxide by the red tide dinoflagellate *Karenia mikimotoi*. *J. Biosci. Bioeng.* 2004, 97, 212–215. [CrossRef]

182. Cho, K.; Kasaoka, T.; Ueno, M.; Basti, L.; Yamasaki, Y.; Kim, D.; Oda, T. Haemolytic activity and reactive oxygen species production of four harmful algal bloom species. *Eur. J. Pharmol.* 2017, 52, 311–319. [CrossRef]

183. Kim, D.; Wencheng, L.; Matsuyma, Y.; Cho, K.; Yamasaki, Y.; Takeshi, S.; Yamaguchi, K.; Oda, T. Extremely high level of reactive oxygen species (ROS) production in a newly isolated strain of the dinoflagellate *Karenia mikimotoi*. *Eur. J. Pharmol.* 2019, 54, 632–640. [CrossRef]

184. Diaz, J.M.; Plummer, S. Production of extracellular reactive oxygen species by phytoplankton: Past and future directions. *J. Plankton Res.* 2018, 40, 655–666. [CrossRef] [PubMed]

185. Kim, D.; Yamaguchi, K.; Oda, T. Nitric oxide synthase-like enzyme mediated nitric oxide generation by harmful red tide phytoplankton, *Chattonella marina*. *Mar. Ecol. Prog. Ser.* 2006, 28, 613–620. [CrossRef]

186. Stamler, J.S. Redox signaling: Nitrosylation and related target interactions of nitric oxide. *Cell* 1994, 78, 931–936. [CrossRef]

187. Lamattina, L.; García-Mata, C.; Graziano, M.; Pagnussat, G. Nitric oxide: The versatility of an extensive signal molecule. *Annu. Rev. Plant Biol.* 2003, 54, 109–136. [CrossRef] [PubMed]

188. Zhang, C.; Czyzmek, K.J.; Shapiro, A.D. Nitric oxide does not trigger early programmed cell death events but may contribute to cell-to-cell signaling governing progression of the *Arabidopsis* hypersensitive response. *Mol. Plant Microbe Interact.* 2003, 16, 962–972. [CrossRef] [PubMed]

189. Yamamoto, A.; Katou, S.; Yoshioka, H.; Doke, N.; Kawakita, K. Involvement of nitric oxide generation in hypersensitive cell death induced by elicitor in tobacco cell suspension culture. *J. Gen. Plant Pathol.* 2004, 70, 85–92. [CrossRef]

190. Lee, T.-Y.; Gotob, N.; Niki, E.; Yokoyama, K.; Tsuzuki, M.; Takeuchi, T.; Karube, I. Chemiluminescence detection of red tide phytoplankton *Chattonella marina*. *Anal. Chem.* 1995, 67, 225–228. [CrossRef]

191. Oda, T.; Nakamura, A.; Okamoto, T.; Ishimatsu, A.; Muramatsu, T. Lectin-induced enhancement of superoxide anion production by red tide phytoplankton. *Mar. Biol.* 1998, 131, 383–390. [CrossRef]

192. Dorantes-Aranda, J.J.; Nichols, P.D.; Waite, T.D.; Hallegraeff, G.M. Strain variability in fatty acid composition of *Chattonella marina* (Raphidophyceae) and its relation to differing ichthyotoxicity toward rainbow trout gill cells. *J. Phycol.* 2013, 49, 427–438. [CrossRef] [PubMed]

193. Yuasa, K.; Shikata, T.; Kitatsui, S.; Yamasaki, Y.; Nishiyama, Y. Extracellular secretion of superoxide is regulated by photosynthetic electron transport in the noxious red-tide-forming raphidophyte *Chattonella antiqua*. *J. Photochem. Photobiol. B Biol.* 2020, 205, 111839. [CrossRef]
194. Kim, D.; Yamasaki, Y.; Yamatogi, T.; Yamaguchi, K.; Matsuayama, Y.; Kang, Y.S.; Lee, Y.; Oda, T. The possibility of reactive oxygen species (ROS)-indipendent toxic effects of *Cochlodinium polykrikoides* on damselfish (*Chromis caeruleus*). *Biosci. Biotechnol. Biochem.* 2009, 73, 613–618. [CrossRef]

195. Sato, E.; Niwano, Y.; Matsuayama, Y.; Kim, D.; Nakashima, T.; Oda, T.; Kohno, M. Some dinophycean red tide plankton species generate a superoxide scavenging substance. *Biosci. Biotechnol. Biochem.* 2007, 71, 704–710. [CrossRef] [PubMed]

196. Vrieling, E.G.; Koeman, R.P.T.; Nagasaki, K.; Ishida, Y.; Pererzak, L.; Gieskes, W.W.C.; Veenhuis, M. *Raphidophycean in southern Brazil.* In *Red Tides, Biology, Environmental Science, and Toxicology*; Okaichi, T., Ed.; Springer: Cham, Switzerland, 2014; pp. 75–84.

197. Hirata, K.; Yoshitomi, S.; Dwi, S.; Iwabe, O.; Mahakant, A.; Polchai, J.; Miyamoto, K. Generation of reactive oxygen species undergoing redox cycle of nostocine A: A cytotoxic violet pigment produced by freshwater cyanobacterium *Nostoc spongiaeforme*. *J. Biotechnol.* 2004, 110, 29–35. [CrossRef]

198. Nathan, C.F.; Root, R.K. Hydrogen peroxide release from mouse peritoneal macrophages: Dependence on sequential activation and triggering. *J. Exp. Med.* 1977, 146, 1648–1662. [CrossRef]

199. Kustka, A.B.; Shaked, Y.; Milligan, A.J.; King, D.W.; Morel, F.M. Extracellular production of superoxide by marine diatoms: Contrasting effects on iron redox chemistry and bioavailability. *Limnol. Oceanogr.* 2005, 50, 1172–1180. [CrossRef]

200. Pick, E.; Keisari, Y. A simple colorimetric method for the measurement of hydrogen peroxide produced by cells in culture. *Anal. Biochem.* 1984, 141, 280–286. [CrossRef]

201. Nathan, C.F.; Root, R.K. Hydrogen peroxide release from mouse peritoneal macrophages: Dependence on sequential activation and triggering. *J. Exp. Med.* 1977, 146, 1648–1662. [CrossRef]

202. Akita, K.; Fujinami, Y.; Nishiyama, Y.; Kotake, T.; Tanaka, R.; Yamasaki, Y. Inhibitory effect of the iron chelator Desferrioxamine (Desferal) on the generation of activated oxygen species by marine phytoplankton using the chemiluminescence probes MCLA and red-CLA. *Limnol. Oceanogr. Methods* 2009, 7, 682–692. [CrossRef]

203. Kim, D.; Oda, T. Production of nitric oxide by marine unicellular red tide phytoplankton, *Chattonella marina*. In *Nitric Oxide in Plants: Metabolism and Role in Stress Physiology*; Springer: Cham, Switzerland, 2014; pp. 75–84.

204. Kim, D.; Kang, Y.S.; Lee, Y.; Yamaguchi, K.; Matsuoka, K.; Lee, K.W.; Choi, K.S.; Oda, T. Detection of nitric oxide (NO) in marine phytoplankters. *J. Biosci. Bioeng.* 2008, 105, 414–417. [CrossRef] [PubMed]

205. Shimada, M.; Shimono, R.; Murakami, T.H.; Yoshimatsu, S.; Ono, C. Red tide, *Chattonella antiqua* reduces cytochrome c from horse heart. In *Red tides: Biology, Environmental Science, and Toxicology*; Okaichi, T., Anderson, D.M., Nemoto, T., Eds.; Elsevier: New York, NY, USA, 1989; pp. 443–446.

206. Fujii, M.; Dang, T.C.; Rose, A.L.; Omura, T.; Waite, T.D. Effect of light on iron uptake by the freshwater cyanobacterium *Microcystis aeruginosa*. *Anal. Biochem.* 2008, 380, 1215–1227. [CrossRef] [PubMed]

207. Odebrecht, C.; Abreu, P.C. Raphidophyeanch in southern Brazil. * Harmful Algae News* 1995, 12, 4.

208. Oda, T.; Moritomi, J.; Kawano, I.; Hamaguchi, S.; Ishimatsu, A.; Muramatsu, T. Catalase-and superoxide dismutase-induced morphological changes and growth inhibition in the red tide phytoplankton *Chattonella marina*. *Biosci. Biotechnol. Biochem.* 1995, 59, 2044–2048. [CrossRef]

209. Kawano, I.; Oda, T.; Ishimatsu, A.; Muramatsu, T. Inhibitory effect of the iron chelator Desferrioxamine (Desferal) on the generation of activated oxygen species by *Chattonella marina*. *Mar. Biol.* 1996, 126, 765–771. [CrossRef]

210. Ahmed, M.S.; Khan, S.; Arakawa, K.; Onoue, Y. Properties of hemagglutinins newly separated from toxic phytoplankton. *Biochim. Biophys. Acta Gen. Subj.* 1995, 1243, 509–512. [CrossRef] [PubMed]

211. Furey, A.; Garcia, J.; O’Callaghan, K.; Lehane, M.; Amandi, M.J.; James, K.J. Brevetoxins: Structure, toxicology and origin. In *Phytoxins: Chemistry and Biochemistry*; Botana, L.M., Ed.; Blackwell Publishing: Hoboken, NJ, USA, 2007; pp. 19–46, ISBN 9780813827001.

212. Okaichi, T. Red tide problems in the Seto Inland Sea, Japan. In *Red Tides, Biology, Environmental Science, and Toxicology*; Okaichi, T., Anderson, D.M., Nemoto, T., Eds.; Elsevier: Amsterdam, The Netherlands, 1989; pp. 137–142.

213. Shikata, T.; Yuasa, K.; Kitatsugi, S.; Sakamoto, S.; Akita, K.; Fujinami, Y.; Nishiyama, Y.; Kotake, T.; Tanaka, R.; Yamasaki, Y. Superoxide production by the red tide-producing *Chattonella marina* complex (Raphidophyceae) correlates with toxicity to aquacultured fishes. *Antioxidants* 2021, 10, 1635. [CrossRef]

214. Rose, A.L.; Salmon, T.P.; Lukondeh, T.; Neilan, B.A.; Waite, T.D. Use of superoxide as an electron shuttle for iron acquisition by the marine cyanobacterium *Lynynga majuscula*. *Environ. Sci. Technol.* 2005, 39, 3708–3715. [CrossRef]

215. Rose, A.L.; Moffett, J.W.; Waite, T.D. Determination of superoxide in seawater using 2-methyl-6-(4-methoxyphenyl)-3,7-dihydroimidazo[1,2-α]pyrazin-3(7H)-one chemiluminescence. *Anal. Chem.* 2008, 80, 1215–1227. [CrossRef]

216. Fujii, M.; Dang, T.C.; Rose, A.L.; Omura, T.; Waite, T.D. Effect of light on iron uptake by the freshwater cyanobacterium *Microcystis aeruginosa*. *Environ. Sci. Technol.* 2011, 45, 1391–1398. [CrossRef]

217. Aquino-Cruz, A.; Band-Schmidt, C.J.; Zenteno-Savin, T. Superoxide production rates and hemolytic activity linked to cellular growth phases in *Chattonella* species (Raphidophyceae) and *Margalefidinium polykrikoides* (Dinophyceae). *J. Appl. Phycol.* 2020, 32, 4029–4046. [CrossRef]

218. Nakamura, A.; Okamoto, T.; Komatsu, N.; Ooka, S.; Oda, T.; Ishimatsu, A.; Muramatsu, T. Fish mucus stimulates the generation of superoxide anion by *Chattonella marina* and *Heterosigma akashiwo*. *Fish. Sci.* 1998, 64, 866–869. [CrossRef]
219. Kim, D.; Watanabe, M.; Nakayasu, Y.; Kohata, K. Production of superoxide anion and hydrogen peroxide associated with cell growth of *Chattonella antiqua*. *Aquat. Microb. Ecol.* 2004, 35, 57–64. [CrossRef]

220. Milne, A.; Davey, M.S.; Worsfold, P.J.; Achterberg, E.P.; Taylor, A.R. Real-time detection of reactive oxygen species generation by marine phytoplankton using flow injection—Chemiluminescence. *Limnol. Oceanogr. Methods* 2009, 7, 706–715. [CrossRef]

221. Diaz, J.M.; Flimmer, S.; Hansel, C.M.; Andeer, P.F.; Saito, M.A.; McLivin, M.R. NADPH-dependent extracellular superoxide production is vital to photophysiology in the marine diatom *Thalassiosira oceanica*. *Proc. Natl. Acad. Sci. USA* 2019, 116, 16448–16453. [CrossRef] [PubMed]

222. Palenik, B.; Zaffiriou, O.C.; Morel, F.M. Hydrogen peroxide production by a marine phytoplankter 1. *Limnol. Oceanogr.* 1987, 32, 1363–1369. [CrossRef]

223. Zhang, T.; Diaz, J.M.; Brighi, C.; Parsons, R.J.; McNally, S.; Apprill, A.; Hansel, C.M. Dark production of extracellular superoxide by the coral *Porites astreoides* and representative symbionts. *Front. Mar. Sci.* 2016, 3, 232. [CrossRef]

224. Halliwell, B.; Gutteridge, J. Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem. J.* 1984, 219, 1. [CrossRef] [PubMed]

225. Oda, T.; Akaike, T.; Hamamoto, T.; Suzuki, F.; Hirano, T.; Maeda, H. Oxygen radicals in influenza-induced pathogenesis and treatment with pyridinamide-conjugated SOD. *Science* 1989, 244, 974–976. [CrossRef]

226. Kim, D.; Oda, T.; Ishimatsu, A.; Muramatsu, T. Isolation and characterization of a mutant strain of *Chattonella marina* with decreased production of superoxide anion. *Biosci. Biotechnol. Biochem.* 1999, 63, 1947–1952. [CrossRef]

227. Matsuyama, Y.; Oda, T. Toxic effects of harmful algal blooms on finfish and shellfish. In *Rev. Fish Biol. Fish.*; Eds.; The Company of Biologists Ltd: London, UK, 1989; pp. 273–278. [CrossRef]

228. Lee, K.; Ishimatsu, A.; Sakaguchi, H.; Oda, T. Cardiac output during exposure to *Chattonella marina* and environmental hypoxia in yellowtail. *Seriola quinquemaculata*. *Mar. Biol.* 2003, 142, 391–397. [CrossRef]

229. Ishimatsu, A.; Tsuchiyama, M.; Yoshida, M.; Sameshima, M.; Pawluk, M.; Oda, T. Effect of *Chattonella* exposure on acid-base status of the yellowtail. *Nippon Suisan Gakkaishi* 1991, 57, 2115–2120. [CrossRef]

230. Tsuchiyama, T.; Ishimatsu, A.; Muramatsu, T. Effect of *Chattonella* exposure on plasma catecholamine levels in the yellowtail. *Nippon Suisan Gakkaishi* 1992, 58, 207–211. [CrossRef]

231. Hishida, Y.; Ishimatsu, A.; Oda, T.; Uchida, S.; Ozaki, M. Effect of *Chattonella* exposure on plasma catecholamine levels in the yellowtail. *Nippon Suisan Gakkaishi* 1992, 58, 207–211. [CrossRef]

232. Kim, D.; Watanabe, M.; Nakayasu, Y.; Kohata, K. Production of superoxide anion and hydrogen peroxide associated with cell growth of *Chattonella antiqua*. *Aquat. Microb. Ecol.* 2004, 35, 57–64. [CrossRef]

233. Lee, K.S.; Ishimatsu, A.; Oda, T. Direct evidence for the reduction of water flow across the gills of yellowtail *Chattonella antiqua*. *Aquat. Microb. Ecol.* 2004, 35, 55–57. [CrossRef] [PubMed]

234. Hale, W.B.; Turner, B.; LaMont, J.T. Oxyradical stimulation of mucus production in the gill of the yellowtail *Chattonella antiqua*. *Limnol. Oceanogr. Methods* 2004, 35, 1947–1952. [CrossRef] [PubMed]

235. LaMont, J.T.; Chantler, E. Oxygen radicals stimulate gallbladder glycoprotein secretion. In *Mucus and Related Topics*; Chantler, E.N., Ratcliffe, N.A., Eds.; The Company of Biologists Ltd: London, UK, 1989; pp. 273–278. [CrossRef]

236. Hiraishi, H.; Terano, A.; Ota, S.; Mutoh, H.; Sugimoto, T.; Razandi, M.; Ivey, K.J. Oxygen metabolites stimulate mucous glycoprotein secretion from cultured rat gastric mucous cells. *Am. J. Physiol.* 1991, 261, G662–G668. [CrossRef] [PubMed]

237. Adler, K.B.; Holden-Stauffer, W.J.; Repine, J.E. Oxygen metabolites stimulate release of high-molecular-weight glycoconjugates by marine phytoplankton using flow injection—Chemiluminescence. *Limnol. Oceanogr. Methods* 2009, 7, 706–715. [CrossRef]

238. Wright, D.T.; Fischer, B.M.; Li, C.; Rochelle, L.G.; Akley, N.J.; Adler, K.B. Oxidant stress stimulates mucin secretion and PLC stimulation by Hepes buffer. *Biochem. J.* 1998, 354, L854–L861. [CrossRef] [PubMed]

239. Shephard, K.L. Functions for fish mucus. *Rev. Fish Biol. Fish.* 1994, 4, 401–429. [CrossRef]

240. Holmgren, S.; Olsson, C. Autonomic control of glands and secretion: A comparative view. *Auton. Neurosci.* 2003, 116, 102–112. [CrossRef]

241. Lesser, M.P. Oxidative stress in marine environments: Biochemistry and physiological ecology. *Annu. Rev. Physiol.* 2006, 68, 253–278. [CrossRef]

242. Simpson, J.A.; Cheeseman, K.H.; Smith, S.E.; Dean, R.T. Free-radical generation by copper ions and hydrogen peroxide. Stimulation by Hepes buffer. *Biochem. J.* 1988, 254, 519–523. [CrossRef]

243. Korshunov, S.S.; Imlay, J.A. A potential role for periplasmic superoxide dismutase in blocking the penetration of external superoxide into the cytosol of Gram-negative bacteria. *Mol. Microbiol.* 2002, 43, 95–106. [CrossRef] [PubMed]

244. Sunda, W.G.; Swift, D.G.; Huntsman, S.A. Low iron requirement for growth in oceanic phytoplankton. *Nature* 1991, 351, 55–57. [CrossRef]

245. Brewer, P.G. Minor elements in sea water. In *Chemical Oceanography*; Riley, J.P., Skirrow, G., Eds.; Academic Press: New York, NY, USA, 1975; pp. 416–490.

246. Tang, J.Y.; Anderson, D.M.; Au, D.W. Hydrogen peroxide is not the cause of fish kills associated with *Chattonella marina*. Cytological and physiological evidence. *Aquat. Toxicol.* 2005, 72, 351–360. [CrossRef] [PubMed]
257. Deken, X.D.; Wang, D.; Many, M.-C.; Costagliola, S.; Libert, F.; Vassart, G.; Dumont, J.E.; Miot, F. Cloning of two human thyroid cDNAs encoding new members of the NADPH oxidase family. J. Biol. Chem. 2000, 275, 23226–23232. [CrossRef] [PubMed]

258. Torres, M.A.; Onouchi, H.; Hamada, S.; Machida, C.; Hammond-Kosack, K.E.; Jones, J.D. Six Arabidopsis thaliana homologues of the human respiratory burst oxidase (gp91phox). Plant J. 1998, 14, 365–370. [CrossRef] [PubMed]

259. Shikata, T.; Takahashi, F.; Nishide, H.; Shigenobu, S.; Kamei, Y.; Sakamoto, S.; Yuasa, K.; Nishiyama, Y.; Yamasaki, Y.; Uchiyama, I. RNA-seq analysis reveals genes related to photoreception, nutrient uptake, and toxicity in a noxious red-tide raphidophyte Chattonella antiqua. Front. Microbiol. 2019, 10, 1764. [CrossRef]

260. Tang, L.B. Neuroprotection by glucose-6-phosphate dehydrogenase and the pentose phosphate pathway. J. Cell. Biochem. 2019, 120, 14285–14295. [CrossRef] [PubMed]

261. Hossain, M.S.; Dietz, K.J. Tuning of redox regulatory mechanisms, reactive oxygen species and redox homeostasis under salinity stress. Front. Plant Sci. 2016, 7, 548. [CrossRef] [PubMed]

262. Marshall, J.A.; Hovenden, M.; Oda, T.; Hallegaard, M.G. Photosynthesis does influence superoxide production in the ichthyotoxic dinoflagellate Cochlodinium polykrikoides. J. Plankton Res. 2002, 24, 1231–1236. [CrossRef]

263. Yuasa, K.; Shikata, T.; Ichikawa, T.; Tamura, Y.; Nishiyama, Y. Nutrient deficiency stimulates the production of superoxide in the noxius red-tide-forming raphidophyte Heterosigma akashiwo. Mar. Biol. 1985, 88, 295–299. [CrossRef]

264. Whyte, J.N.C.; Haigh, N.; Ginther, N.G.; Keddy, L.J. First record of blooms of Cochlodinium sp. (Gymnodiniales, Dinophyceae) causing mortality to aquacultured salmon on the west coast of Canada. Physiologia 2001, 40, 298–304. [CrossRef]

265. Inoue, I.; Yamaguchi, M.; Watanabe, M. Ecophysiology, life cycle, and bloom dynamics of an unarmored dinoflagellate Chattonella antiqua. Bull. Jpn. Soc. Sci. Fish. 1989, 55, 295–299. [CrossRef]

266. Fletcher, T.C. The identification of nonspecific humoral factors in the plaice (Pleuronectes platessa L.). Dev. Biol. Stand. 1981, 49, 321–327.

267. Imai, I.; Yamaguchi, M.; Watanabe, M. Ecophysiology, life cycle, and bloom dynamics of an unarmored dinoflagellate Chattonella antiqua. Bull. Jpn. Soc. Sci. Fish. 1989, 55, 295–299. [CrossRef]

268. Mulholland, M.R.; Morse, R.E.; Boneillo, G.E.; Bernhardt, P.W.; Filippino, K.C.; Procise, L.A.; Blanco-Garcia, J.L.; Marshall, H.G.; Egerton, T.A.; Hunley, W.S.; et al. Understanding causes and impacts of the dinoflagellate, Cochlodinium polykrikoides blooms in the Chesapeake Bay. Estuar. Coasts 2009, 32, 734–747. [CrossRef]

269. Whyte, J.N.C.; Haigh, N.; Ginther, N.G.; Keddy, L.J. First record of blooms of Cochlodinium sp. (Gymnodiniales, Dinophyceae) causing mortality to aquacultured salmon on the west coast of Canada. Physiologia 2001, 40, 298–304. [CrossRef] [PubMed]

270. Kudela, R.M.; Gobler, C.J. Harmful dinoflagellate blooms caused by Cochlodinium sp: Global expansion and ecological strategies facilitating bloom formation. Harmful Algae 2012, 14, 71–86. [CrossRef] [PubMed]

271. Yamaguchi, M.; Watanabe, M.; Imae, I.; Takahashi, F.; Nishide, H.; Shigenobu, S.; Kamei, Y.; Sakamoto, S.; Yuasa, K.; Nishiyama, Y.; Yamasaki, Y.; Uchiyama, I. RNA-seq analysis reveals genes related to photoreception, nutrient uptake, and toxicity in a noxious red-tide raphidophyte Chattonella antiqua. Front. Microbiol. 2019, 10, 1764. [CrossRef]

272. Tang, L.B. Neuroprotection by glucose-6-phosphate dehydrogenase and the pentose phosphate pathway. J. Cell. Biochem. 2019, 120, 14285–14295. [CrossRef] [PubMed]
274. Gobler, C.J.; Berry, D.L.; Anderson, O.R.; Burson, A.; Koch, F.; Rodgers, B.S.; Moore, L.K.; Goleski, J.A.; Allam, B.; Bowser, P.; et al. Characterization, dynamics, and ecological impacts of harmful *Gymnodinium polykrikoides* blooms on eastern Long Island, NY, USA. *Harmful Algae* **2008**, *7*, 293–307. [CrossRef]

275. Kim, H.G. *Gymnodinium polykrikoides* blooms in Korean coastal waters and their mitigation. In *Harmful Algae*; Reguera, B., Blanco, J., Fernandez, M.L., Wyatt, T., Eds.; Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO: Santiago de Compostela, Spain, 1998; pp. 227–228.

276. Gárate-Lázarraga, I.; López-Cortes, D.J.; Bustillos-Guzmán, J.J.; Hernández-Sandoval, F. Blooms of *Gymnodinium polykrikoides* (*Gymnodiniaceae*) in the Gulf of California, Mexico. *Rev. Biol. Trop.* **2004**, *52*, 51–58.

277. Kim, C.S.; Lee, S.G.; Kim, H.G. Use of dissolved inorganic and organic phosphorus by axenic and nonaxenic clones of *Cochlodinium polykrikoides*. *J. Plankton Res.* **2006**, *28*, 151–157. [CrossRef]

278. Shin, Y.K.; Nam, S.-E.; Kim, W.J.; Seo, D.Y.; Kim, Y.-J.; Rhee, J.-S. Red tide dinoflagellate *Gymnodinium polykrikoides* induces significant oxidative stress and DNA damage in the gill tissue of the red seabream *Pagrus major*. *Harmful Algae* **2019**, *86*, 37–45. [CrossRef] [PubMed]

279. Li, X.; Yan, T.; Lin, J.; Yu, R.; Zhou, M. Detrimental impacts of the dinoflagellate *Karenia mikimotoi* in Fujian coastal waters on typical marine organisms. *Harmful Algae* **2017**, *61*, 1–12. [CrossRef]

280. Koizumi, Y.; Uchida, T.; Honjo, T. Diurnal vertical migration of *Gymnodinium mikimotoi* during a red tide in Hoketsu Bay, Japan. *J. Plankton Res.* **1996**, *18*, 289–294. [CrossRef]

281. Yamaguchi, M.; Honjo, T. Effects of temperature, salinity and irradiance on the growth of the noxious red tide flagellate *Gymnodinium nagasakense* (*Dinophyceae*). *Nippon Suisan Gakkaishi* **1989**, *55*, 2029–2036. [CrossRef]

282. Lei, Q.Y.; Lu, S.H. Molecular ecological responses of the dinoflagellate *Karenia mikimotoi* to phosphate stress. *Harmful Algae* **2011**, *12*, 39–45. [CrossRef]

283. Richardson, B.; Corcoran, A.A. Use of dissolved inorganic and organic phosphorus by axenic and nonaxenic clones of *Karenia brevis* and *Karenia mikimotoi*. *Harmful Algae* **2015**, *48*, 30–36. [CrossRef]

284. Yoshimatsu, S. Long-term variation of phytoplankton in southern part of Harimanada. *Plankton Div. Plankton Soc. Jpn.* 1966.

285. Backus, D.; Davidoff, L.; Hackney, K.; McMillan, T.; Norkus, J.; Tanaka, Y. Effect of *Gymnodinium polykrikoides* on the larvae of the Japanese silver herring *C. japonicus*. *Harmful Algae* **2004**, *51*, 231–238. [CrossRef]

286. Sun, J.; Wang, X.D.; Song, S.Q. Selective feeding of *Gyrodinium aureolum* (Dinophyta) on *Chrysochromulina polylepis* components of *Gyrodinium aureolum* *Dinophyceae*. *Bull. Fac. Fish. Nagasaki Univ.* **1961**, *21*, 67–101.

287. Kim, H.G. Cochlodinium polykrikoides blooms in Korean coastal waters and their mitigation. In *Harmful Algae*; Reguera, B., Blanco, J., Fernandez, M.L., Wyatt, T., Eds.; Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO: Santiago de Compostela, Spain, 1998; pp. 227–228.

288. Gómez-Álvarez, I.; López-Cortes, D.J.; Bustillos-Guzmán, J.J.; Hernández-Sandoval, F. Blooms of *Gymnodinium polykrikoides* (*Gymnodiniaceae*) in the Gulf of California, Mexico. *Rev. Biol. Trop.* **2004**, *52*, 51–58.

289. Kim, C.S.; Lee, S.G.; Kim, H.G. Use of dissolved inorganic and organic phosphorus by axenic and nonaxenic clones of *Cochlodinium polykrikoides*. *J. Plankton Res.* **2006**, *28*, 151–157. [CrossRef]

290. Gentien, P.; Lunven, M.; Lazure, P.; Youenou, A.; Crassous, M.P. Motility and autotoxicity in *Gymnodinium mikimotoi* (*Gymnodiniaceae*). *Mol. Ecol. Res.* **2003**, *3*, 592–598. [CrossRef] [PubMed]

291. Jenkinson, I.R.; Arzul, G. Mitigation by cysteine compounds of rheotoxicity, cytotoxicity and fish mortality caused by the toxic red tide dinoflagellate, *Karenia mikimotoi* (*Kareniaceae* (*Dinophyta*): Chemotaxonomy and putative lipid phycotoxins. *J. Phycol.* **2007**, *43*, 101–111. [CrossRef]

292. Davidson, K.; Miller, P.; Wilding, T.A.; Shutler, J.; Bresnan, E.; Kennington, K.; Swan, S. A large and prolonged bloom of *Karenia mikimotoi* (*Kareniaceae* (*Dinophyta*): Chemotaxonomy and putative lipid phycotoxins. *J. Phycol.* **2007**, *43*, 101–111. [CrossRef]

293. Sellem, F.; Pesando, D.; Bodenec, G.; Abed, A.E.; Girard, J.P. Toxic effects of *Gymnodinium cf. mikimotoi* unsaturated fatty acids to gametes and embryos of the sea urchin *Paracentrotus lividus*. *Water Res.* **2000**, *34*, 550–556. [CrossRef]

294. Satake, M.; Shoji, M.; Oshima, Y.; Naoki, H.; Fujita, T.; Yasumoto, T. Gymnocin-A, a cytotoxic polyether from the notorious red tide dinoflagellate, *Karenia mikimotoi* (*Kareniaceae* (*Dinophyta*): Chemotaxonomy and putative lipid phycotoxins. *J. Phycol.* **2004**, *40*, 592–598. [CrossRef] [PubMed]

295. Satake, M.; Shoji, M.; Oshima, Y.; Naoki, H.; Fujita, T.; Yasumoto, T. Gymnocin-B with the largest contiguous polyether rings from the red tide dinoflagellate, *Karenia mikimotoi*. *Tetrahedron Lett.* **2002**, *43*, 5829–5832. [CrossRef]

296. Satake, M.; Tanaka, Y.; Ishikura, Y.; Oshima, Y.; Naoki, H.; Yasumoto, T. Gymnocin-B with the largest contiguous polyether rings from the red tide dinoflagellate, *Karenia mikimotoi*. *Tetrahedron Lett.* **2002**, *43*, 5829–5832. [CrossRef]

297. Matsuyama, Y. Effects of harmful dinoflagellates, *Gymnodinium mikimotoi* and *Heterocapsa circularisquama*, red-tide on filtering rate of bivalve mollusks. *Fish. Sci.* **1999**, *65*, 248–253. [CrossRef]

298. Basti, L.; Nagai, S.; Go, J.; Okano, S.; Nagai, K.; Watanabe, R.; Suzuki, T.; Tanaka, Y. Differential inimical effects of *Alexandrium* spp. and *Karenia* spp. on cleavage, hatching, and two larval stages of Japanese pearl oyster *Pinctada fucata martensii*. *Harmful Algae* **2015**, *43*, 1–12. [CrossRef]
301. Cohen, M.S.; Metcalf, J.A.; Root, R.K. Regulation of oxygen metabolism in human granulocytes: Relationship between stimulus binding and oxidative response using plant lectins as probes. *Blood* 1980, 55, 1003–1009. [CrossRef]

302. Kayashima, K.; Onoue, K.; Nakagawa, A.; Minakami, S. Superoxide anion-generating activities of macrophages as studied by using cytochalasin E and lectins as synergistic stimulants for superoxide release. *Microbiol. Immun.* 1980, 24, 449–461. [CrossRef]

303. Zou, Y.; Yamasaki, Y.; Matsuyama, Y.; Yamaguchi, K.; Honjo, T.; Oda, T. Possible involvement of haemolytic activity in the contact-dependent lethal effects of the dinoflagellate *Karenia mikimotoi* on the rotifer *Brachionus plicatilis*. *Harmful Algae* 2010, 9, 367–373. [CrossRef]

304. Zou, Y.; Kim, D.; Yagi, M.; Yamasaki, Y.; Kurita, J.; Iida, T.; Matsuyama, Y.; Yamaguchi, K.; Honjo, T.; Oda, T. Application of LDH-release assay to cellular-level evaluation of the toxic potential of harmful algal species. *Biosci. Biotechnol. Biochem.* 2013, 77, 345–352. [CrossRef][PubMed]

305. Li, X.D.; Wang, X.Y.; Xu, M.E.; Jiang, Y.; Yan, T.; Wang, X.C. Progress on the usage of the rotifer *Brachionus plicatilis* in marine ecotoxicology: A review. *Aquat. Toxicol.* 2020, 229, 105678. [CrossRef]

306. Kim, D.; Li, W.; Matsuyama, Y.; Matsu, O.; Yagi, M.; Cho, K.; Yamamichi, S.; Yamaguchi, K.; Oda, T. Strain-dependent lethal effects on abalone and haemolytic activities of the dinoflagellate *Karenia mikimotoi*. *Aquaculture* 2020, 520, 734953. [CrossRef]

307. Kikuchi, K.; Nagano, T.; Hayakawa, H.; Hirata, Y.; Hirobe, M. Detection of nitric oxide production from perfused organ by luminol-H$_2$O$_2$ system. *Anal. Chem.* 1993, 65, 1794–1799. [CrossRef][PubMed]

308. Pfeiffer, S.; Leopold, E.; Hemmens, B.; Schmidt, K.; Werner, E.R.; Mayer, B. Interference of carboxy-PTIO with nitric oxide- and peroxynitrite-mediated reactions. *Free Radic. Biol. Med.* 1997, 22, 787–794. [CrossRef]

309. Green, L.C.; Wagner, D.A.; Glogowski, J.; Skipper, P.L.; Wishnok, J.S.; Tannenbaum, S.R. Analysis of nitrate, nitrite, and (15N) nitrate in biological fluids. *Anal. Biochem.* 1982, 126, 131–138. [CrossRef]

310. Taylor, M.B.; Christian, K.G.; Patel, N.; Churchwell, K.B. Methemoglobinemia: Toxicity of inhaled nitric oxide therapy. *Pediatr. Crit. Care Med.* 2001, 2, 99–101. [PubMed]

311. Grossel, M.; Jensen, F.B. NO$^-$ uptake and HCO$_3^-$ excretion in the intestine of the European flounder (*Platichthys flesus*). *J. Exp. Biol.* 1999, 202, 2103–2110. [CrossRef]
330. Vedel, N.E.; Korsgaard, B.; Jensen, F.B. Isolated and combined exposure to ammonia and nitrite in rainbow trout (Oncorhynchus mykiss): Effects on electrolyte status, blood respiratory properties and brain glutamine: Glutamate concentrations. *Aquat. Toxicol.* 1998, 41, 325–342. [CrossRef]

331. Shen, M.; Xu, J.; Chiang, M.W.; Au, D.W. Unravelling the pathway of respiratory toxicity in goldlined seabream (Rhabdosargus sarba) induced by the harmful alga *Chattonella marina*. *Aquat. Toxicol.* 2011, 104, 185–191. [CrossRef] [PubMed]

332. Kumar, A.; Castellano, I.; Patti, F.P.; Palumbo, A.; Buia, M.C. Nitric oxide in marine photosynthetic organisms. *Nitric Oxide* 2015, 47, 34–39. [CrossRef]

333. Kuroda, A.; Nakashima, T.; Yamaguchi, K.; Oda, T. Isolation and characterization of light-dependent hemolytic cytotoxin from harmful red tide phytoplankton *Chattonella marina*. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* 2005, 141, 297–305. [CrossRef] [PubMed]

334. Kim, D. Radical scavengers in red tide plankton, *Chattonella marina*. *Bull. Fac. Fish. Nagasaki Univ.* 2001, 82, 93–97.

335. Sato, E.; Niwano, Y.; Mokudai, T.; Kohno, M.; Matsuyama, Y.; Kim, D.; Oda, T. A discrepancy in superoxide scavenging activity between the ESR-spin trapping method and the luminol chemiluminescence method. *Biosci. Biotechnol. Biochem.* 2007, 71, 1505–1513. [CrossRef] [PubMed]

336. Niwano, Y.; Sato, E.; Kohno, M.; Matsuyama, Y.; Kim, D.; Oda, T. Antioxidant properties of aqueous extracts from red tide plankton cultures. *Biosci. Biotechnol. Biochem.* 2007, 71, 1145. [CrossRef] [PubMed]

337. Shimasaki, Y.; Mukai, K.; Takai, Y.; Qiu, X.; Oshima, Y. Recent progress in the study of peroxiredoxin in the harmful algal Bloom species *Chattonella marina*. *Antioxidants* 2021, 10, 162. [CrossRef] [PubMed]