



Inhibition of harmful algal blooms caused by *Aureococcus anophagefferens* (Pelagophyceae) using native (*Gracilaria tikvahiae*) and invasive (*Dasysiphonia japonica*) red seaweeds from North America

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Abstract

Harmful algal blooms (HABs) caused by the pelagophyte *Aureococcus anophagefferens* have spread globally and are a threat to coastal ecosystems. Although some HAB mitigation techniques such as algal biocides can be effective, many such approaches can have negative consequences on the environment. Therefore, it is important to investigate alternative HAB mitigation approaches that are ecologically safe and commercially viable. Here, we examine the effects of two rhodophyte seaweeds, *Gracilaria tikvahiae* and *Dasysiphonia japonica*, on *A. anophagefferens*. During experiments, multiple *A. anophagefferens* strains were co-cultured with densities of seaweed encompassing a range of environmental and aquaculture settings (0.25–3.00 g L⁻¹). The co-effects of nutrients, heterotrophic bacteria and pH were also assessed, and *A. anophagefferens* cell concentrations and photosynthetic efficiency were quantified. In nearly all experiments, *G. tikvahiae* and *D. japonica* significantly reduced *A. anophagefferens* cell concentrations in a dose-dependent manner, with the effectiveness of each seaweed depending primarily on both *A. anophagefferens* and seaweed densities. The algicidal effects of *D. japonica* were significantly stronger than *G. tikvahiae* and other algicidal seaweeds (e.g., *Ulva* spp.). Allelopathy was the most potent mechanism for inhibition of *A. anophagefferens*; heterotrophic bacteria, nutrient limitation and elevated pH appeared to have lesser co-effects. These findings, in conjunction with the well-known nutrient removal capacity of seaweeds, suggest that the temporal and spatial dynamics of these rhodophytes may influence brown tides in shallow estuaries and that the use of seaweeds such as *G. tikvahiae* in aquaculture may be a promising mitigation strategy for this and other HABs in coastal ecosystems.

Keywords Brown tide · Harmful algal blooms · Macroalgae · Allelopathy · Aquaculture

Introduction

While harmful algal blooms (HABs) are natural phenomena that have occurred throughout documented history, the public health and economic impacts of such events have increased in frequency, severity and geographical distribution over the past two decades (Anderson et al. 2012, 2021). HABs can be a public health threat due to the synthesis of potent biotoxins (Van Dolah 2000) and can also threaten fisheries, aquatic habitats and aquatic ecosystems

(Sunda et al. 2006). There has been a statistically significant increase in all HAB events combined in the USA since 1990 (Anderson et al. 2021) and the coastal northeastern USA is emblematic of these trends, as, over the past several decades, it has increasingly experienced multiple HABs that had previously been unknown in the region (Anderson et al. 2008, 2021). One such HAB in this region is caused by the picoplankton, *Aureococcus anophagefferens*, colloquially known as “brown tide.” Since the 1980s, intense brown tide events ($> 1 \times 10^6$ cells mL⁻¹) have occurred annually in coastal embayments in the northeastern USA, threatening coastal ecosystems and shellfish industries (Gobler and Sunda 2012). The onset of severe brown tides in New York (NY) nearly eliminated Long Island’s bay scallop (*Argopecten irradians*) population by causing extensive recruitment

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failure, a US\$3.3 million annual fishery (Hoagland et al. 2002), and has caused mass mortality of the former largest fishery in NY, the hard clam (*Mercenaria mercenaria*) (Gobler and Sunda 2012). Field observations indicate that *A. anophagefferens* blooms occur when dissolved organic nitrogen (DON) concentrations are high and dissolved inorganic nitrogen (DIN) concentrations are low (Gobler et al. 2002; Gobler and Sunda 2012). In addition, anthropogenic nutrient loading on Long Island has steadily risen over the last few decades (SCSWP 2020), in part due to time-lagged groundwater discharge enriched in nitrogen from wastewater (LaRoche et al. 1997; SCSWP 2020). This nutrient loading, especially when combined with slow estuarine flushing, can promote brown tides (Gobler et al. 2019).

During the past decade, there have been a series of observations that certain seaweeds can inhibit the growth of specific HAB-causing phytoplankton (Xu and He 2006; Yang et al. 2015). Certain species of rhodophytes (red algae) may inhibit the growth of HABs by creating competition for nutrients or secreting allelopathic compounds (Tang et al. 2015). For example, multiple species of *Gracilaria* endemic to Asian waters have been shown to inhibit the growth of multiple HABs via allelopathy (Ye and Zhang 2013; Ye et al. 2014; Yang et al. 2015). *Gracilaria tikvahiae* is distributed across the western Atlantic Ocean; its range overlaps spatially and temporally with many significant HABs in the northeast USA, including *A. anophagefferens* brown tides. Although Lapointe (2008) showed that Asian species of *Gracilaria* can outcompete phytoplankton for essential nutrients in estuarine environments, there are no studies evaluating the effects of Atlantic species of *Gracilaria* on HAB-causing phytoplankton. In addition to *G. tikvahiae*, *Dasyisiphonia japonica* is a rhodophyte native to the western Pacific Ocean that invaded European waters in 1984 (Sjötun et al. 2008) and has since spread to the western Atlantic Ocean, being first documented in the waters around Rhode Island in 2007 (Schneider 2010). *Dasyisiphonia japonica* experiences enhanced growth compared to other red macroalgae when exposed to elevated $p\text{CO}_2$ and nutrient levels, which may allow it to outcompete other algae in eutrophic estuaries (Young and Gobler 2021). The potential effects of *D. japonica* on HAB phytoplankton, including *A. anophagefferens*, have never been investigated.

Given the observed inhibitory effects of seaweeds on multiple HAB phytoplankton species, seaweed cultivation is a potential technique for HAB mitigation and control, especially in the context of integrated multi-trophic aquaculture (IMTA). While members of the algae phylum Chlorophyta (i.e., *Ulva*) exhibit the potential to outcompete harmful phytoplankton via allelopathy, their overgrowth, known as “green tides,” has been considered dangerous HABs in their own right and has limited economic value (Ye et al. 2011; Smetacek and Zingone 2013), therefore not being an ideal

aquaculture species (Tang and Gobler 2011). In contrast, rhodophytes have been recognized as practical candidates for mitigating HABs (Yang et al. 2015). In contrast, cultivated rhodophytes produce higher yields than other algal phyla and can provide economically valuable extracts, including agar, which is produced by the majority of gracillariid species (FAO 2020). If grown in a large-scale aquaculture system, the cultivation of rhodophytes may have significant benefits to co-cultured organisms and entire ecosystems via the reduction of nutrient levels and, potentially, the inhibition of HABs (Neori et al. 2004; Yang et al. 2015; Duarte et al. 2017).

The objective of this study, therefore, was to assess how different densities of *G. tikvahiae* or *D. japonica* affect the growth and abundance of the brown tide-forming pelagophyte, *A. anophagefferens*. A series of experiments were performed to quantify changes in concentrations of *A. anophagefferens* following exposure to *G. tikvahiae* and *D. japonica*. Several densities and durations of exposure to both seaweeds were evaluated in conjunction with different concentrations of *A. anophagefferens*, populations of *A. anophagefferens* (clonal cultures and bloom populations) and differing environmental conditions (e.g., varying levels of nutrients, pH, and antibiotics) to refine the understanding of how these rhodophytes may alter growth rates, cell concentrations and photosynthetic efficiency of *A. anophagefferens*.

Methods

Preparation of cultures and seaweed

Seaweeds were collected from shallow regions of eastern Shinnecock Bay, NY, USA (40.85° N, 72.50° W; Supplementary Fig. S1) during low tide. Permission to access the water and collect the water and seaweed was received from the Southampton Town Trustees, Southampton, NY, USA, who hold jurisdiction over Shinnecock Bay. Collections targeted well-pigmented, robust fronds of *Dasyisiphonia japonica* and *Gracilaria tikvahiae* that were transferred to dark, temperature-controlled containers filled with seawater and returned to the Stony Brook Southampton Marine Science Center within 15 min of collection. For branching macroalgae (*G. tikvahiae* and *D. japonica*), individual fronds of approximately 5 cm in length were cut from the main thalli with a razor blade to obtain the approximate size required and spun in a salad spinner to remove debris and epiphytes (Young and Gobler 2016; Sylvers and Gobler 2021). Samples were then extensively rinsed with filtered (0.2 µm) seawater and placed into the salad spinner a second time to further remove debris, epiphytes and excess seawater. All samples were weighed on an A&D EJ300 digital scale (± 0.01 g) to obtain initial fresh weights in grams.

Samples were further trimmed with a razor blade to the target wet weight. To prevent desiccation, all samples were kept in individual, 100 mL filtered (0.2 µm) seawater-filled containers after spinning prior to use in experiments. Cultures of *Aureococcus anophagefferens* (strains CCMP 1850, CCMP1707, CCMP1984) were maintained in exponential phase growth in GSe medium at 100 µmol photons m⁻² s⁻¹ with a 14:10-h light–dark cycle at 21°C. Unless otherwise noted, experiments were performed with strain CCMP 1850.

Culture experiments

This study relied, in part, on previously published methodologies aimed at testing the growth inhibiting effects of macroalgae on phytoplankton using different concentrations of nutrients (Yang et al. 2015; Sylvers and Gobler 2021). Laboratory experiments were conducted using cultures of *A. anophagefferens* at levels of brown tide representative of peak-bloom and sub-bloom conditions (1 × 10⁶ cells mL⁻¹ and 5 × 10⁵ cells mL⁻¹, respectively; (Gobler and Sunda 2012)) that were exposed to increasing levels of *D. japonica* or *G. tikvahiae*, ranging from 0.25 to 3.00 g L⁻¹, which is representative of full bottom coverage in a shallow estuary (up to 1 m³; Tang and Gobler 2011) and dense deployment in an aquaculture setting (3 g L⁻¹; Neori et al. 2004; Sylvers and Gobler 2021), respectively. Experiments were established in 250 mL polyethylene beakers filled with 0.2 µm-filtered seawater with light levels of 100 µmol photons m⁻² s⁻¹ on a 14:10-h light–dark cycle, and temperatures set at 21°C. Filtered seawater (0.2 µm) was added to dilute the brown tide cultures and achieve desired concentrations. Since concentrations of nutrients in filtered seawater (FSW) were low (< 5 µM dissolved inorganic nitrogen (NO₃⁻ + NO₂⁻ + NH₄⁺) and < 1 µM PO₄³⁻), nitrate (50 µM) and phosphate (3 µM) were added to all flasks at the commencement of experiments to assure nutrient replete conditions, unless otherwise noted. Experiments lasted two-to-four days and densities of the brown tide alga, *A. anophagefferens*, were quantified on a Beckman Coulter Cytoflex after preservation with 1% glutaraldehyde. The pH and temperature of each experimental vessel was measured with a Thermoscientific Orion Star A321.

Assessing the mechanisms of rhodophyte inhibition of *A. anophagefferens*

Beyond allelopathic chemicals, additional growth inhibiting mechanisms of macroalgae may include nutrient competition (Onuf et al. 1977), pH elevation (Hansen 2002) and possible algicidal activity of seaweed biofilm-attached heterotrophic bacteria (Imai et al. 2006; Imai 2015). To explore these mechanisms, experiments were performed as outlined above with minor modification. To assess the effects of nutrients,

A. anophagefferens and macroalgae were co-cultured with and without nutrients added. To assess the effects of bacteria, *A. anophagefferens* and macroalgae were co-cultured with and without 1% antibiotic solution (10,000 I.U. penicillin and 10,000 µg mL⁻¹ streptomycin) to minimize bacterial growth. Antibiotics were added initially and after 48 h and *A. anophagefferens* was stocked with an initial concentration of 1 × 10⁶ cells mL⁻¹ for this experiment. To further understand the potential role of elevated pH in the growth inhibiting effects of the rhodophytes on *A. anophagefferens*, experiments were performed with ~ 3 × 10⁵ *A. anophagefferens* cells mL⁻¹ in the absence of macroalgae whereby the pH of GSe media was buffered to initial pH values of 8.00, 8.25, 8.50 and 9.00—levels corresponding to pH increases caused by seaweeds observed in prior experiments—using 1 mM sodium carbonate. pH was measured daily among cultures using an Orion Star A321 pH Meter. Concentrations of nitrate, ammonium and orthophosphate were measured on a Lachat Autoanalyzer 8500.

Experiments to refine the understanding of rhodophyte inhibition of *A. anophagefferens*

To compare the growth inhibiting effects of the rhodophytes to the previously established effects of *Ulva* spp. (Tang and Gobler 2011), an experiment was performed as described above whereby *G. tikvahiae*, *D. japonica* and *Ulva* spp. were each added to triplicate flasks of *A. anophagefferens* culture at 1 g L⁻¹ and was monitored for 48 h. Given *A. anophagefferens* is known to be promoted by organic nitrogen and specifically urea (Gobler and Sunda 2012), an experiment was performed comparing the effects of *D. japonica* on *A. anophagefferens* cultures grown on nitrate or urea (50 µM N for each) and 3 µM orthophosphate. To determine the extent to which the inhibitory effects of *G. tikvahiae* and *D. japonica* on *A. anophagefferens* were generalized across *A. anophagefferens* populations, experiments were performed with strains CCMP1707, CCMP1984, and CCMP1850 at 4 × 10⁵ cells mL⁻¹ exposed to 1 g L⁻¹ of *D. japonica* or 1 g L⁻¹ *G. tikvahiae*.

Field experiments during a brown tide bloom

A field experiment was conducted during a brown tide bloom in Great South Bay, NY, USA (40.7244 °N, 73.0766 °W) in June 2020. *G. tikvahiae*, and *D. japonica* were co-cultured with a wild population of *A. anophagefferens* in 30, acid-washed, 1-L polycarbonate flasks, containing 800 mL of bloom water. Experimental flasks were incubated under 100 µmol photons m⁻² s⁻¹ with a 14:10-h light–dark cycle at 21°C at both ambient and elevated dissolved inorganic phosphorus and nitrogen (DIP and DIN, respectively) levels (3 µM orthophosphate and 50 µM nitrate for elevated, no

amendment for ambient), as well as two different levels of *G. tikvahiae* and *D. japonica*, 1 g L⁻¹ or 3 g L⁻¹. *Aureococcus anophagefferens* cell concentrations were quantified initially and after 24 h using a Beckman Coulter Cytoflex flow cytometer after preservation with glutaraldehyde and staining with a monoclonal fluorescent antibody following the methods of Stauffer et al. (2008). In addition to *A. anophagefferens*, cell concentrations of phycocyanin- and phycoerythrin-containing cyanobacteria were also quantified via flow cytometry based on their auto-fluorescent patterns (Kang et al. 2015).

Post-experimental analyses

ANOVAs were performed using R 4.1.0 within RStudio 1.3.1073 on the final timepoint of each experiment to assess treatment effects on *A. anophagefferens* cell concentrations. One-way ANOVAs were performed to assess differences in *A. anophagefferens* concentrations when the main treatment effect was the level of seaweed (g L⁻¹), pH level or seaweed species. Two-way ANOVAs were performed to assess changes in cells concentrations when the main treatment effects were nutrients (ambient or enhanced) and level of seaweed (g L⁻¹), the presence of antibiotics and level of seaweed (g L⁻¹), type of nutrients (DIN/DON) and level of seaweed (g L⁻¹) or seaweed species and level of seaweed (g L⁻¹). A three-way ANOVA was performed to assess significant differences in *A. anophagefferens* concentrations during the field experiment when the experimental parameters were level of seaweed (g L⁻¹), seaweed species and nutrients (ambient or enhanced). Tukey's HSD test was utilized to resolve significant differences among treatments. The significance level was set at 0.05 for all tests unless otherwise stated. Non-normal data or data with unequal variance were transformed or examined with non-parameter tests (e.g., Kruskal Wallis).

Results

Gracilaria tikvahiae

In the experiment where high densities of *A. anophagefferens* (1×10^6 cells mL⁻¹) were exposed to high (3 g L⁻¹) and low (1 g L⁻¹) levels of *G. tikvahiae*, with and without nutrient amendment (Table 1), dose of *G. tikvahiae* was a significant factor ($p < 0.001$), however nutrient level was not (Two-way ANOVA; $p > 0.05$; Fig. 1A; Supplementary Table S1). In the high *A. anophagefferens* level treatment (10^6 cells mL⁻¹), cell concentrations were significantly lower than the control in the 3 g L⁻¹ treatment after only 24 h (Tukey HSD; $p < 0.05$; Fig. 1A; Supplementary Table S3) while it took 48 h for the 1 g L⁻¹ treatment to reduce levels

significantly below the control treatment, regardless of nutrient levels (Tukey HSD; $p < 0.05$; Fig. 1A; Supplementary Table S3). In addition, cell concentrations in the 3 g L⁻¹ treatment were significantly lower than 1 g L⁻¹ at each time points (Tukey HSD; $p < 0.05$; Fig. 1A; Supplementary Table S3). After 48 h in the high *A. anophagefferens* cell density experiment, control cell concentrations were 1.8 and 2.0×10^6 cells mL⁻¹ in the low and high nutrient treatments, while the levels in the *G. tikvahiae* treatment were one order of magnitude lower (Fig. 1A).

In the experiment where low densities of *A. anophagefferens* (5×10^5 cells mL⁻¹) were exposed to high (3 g L⁻¹) and low (1 g L⁻¹) levels of *G. tikvahiae*, with and without nutrient amendment (Table 1), dose of *G. tikvahiae*, nutrient level, and the interaction between nutrients and the level of macroalgae were all significant (Two-way ANOVA; $p < 0.05$ for all; Fig. 1B; Supplementary Table S2). While the elevated nutrients control cultures significantly achieved higher densities (1.1×10^6 cells mL⁻¹) than the control culture without nutrients (0.8×10^6 cells mL⁻¹), cell concentrations in treatments with macroalgae added were lower and nearly identical in the high and low nutrient treatments at each level of macroalgae, accounting for the significant interaction (Fig. 1B). After 24 h, *A. anophagefferens* cell concentrations significantly lower than the control in the 3 g L⁻¹ treatments regardless of nutrients but were no different than the control in either 1 g L⁻¹ treatment (Tukey HSD; $p < 0.05$; Fig. 1B; Supplementary Table S3). After 48 h, the control cell concentrations were 0.8 and 1.1×10^6 cells mL⁻¹ in the low and high nutrient treatments, respectively, while the levels in the 1 g L⁻¹ *G. tikvahiae* treatment were $\sim 5 \times 10^4$ cells mL⁻¹ and cells were undetectable in the 3 g L⁻¹ treatment, all levels significantly lower than the control (Tukey HSD; $p < 0.05$; Fig. 1B; Supplementary Table S3). In addition, cell concentrations in the 3 g L⁻¹ treatment were significantly lower than the 1 g L⁻¹ treatment (Tukey HSD; $p < 0.05$; Fig. 1B; Supplementary Table S2).

In the next two experiments, *A. anophagefferens* at high and low cell concentrations (1×10^6 and 5×10^5 mL⁻¹) were exposed to lower levels of *G. tikvahiae*: 0.00 g L⁻¹ (control), 0.25, 0.50, 0.75, and 1.00 g L⁻¹, with and without nutrients supplementation (Table 1). When nutrients were added, dose of *G. tikvahiae* was a significant factor at both high and low *A. anophagefferens* cell concentrations (One-way ANOVA; $p < 0.001$; Fig. 2; Supplementary Tables S4 and S5) whereas in the experiment without nutrient supplementation (Fig. 3), dose of *G. tikvahiae* was a significant factor only at low *A. anophagefferens* cell concentrations (One-way ANOVA; $p < 0.05$; Fig. 3; Supplementary Tables S7 and S8). In the experiment with nutrients added, within the high and low *A. anophagefferens* treatments, cell concentrations in the 1.00 g L⁻¹ *G. tikvahiae* treatment were significantly lower than any other treatments after 72 h (Tukey HSD; $p < 0.05$;

Table 1 Experiments with their respective species cultured, cell concentrations, and macroalgal densities. In the ‘Experimental Conditions’ column, ambient nutrients refer to background levels (<5 μM and <1 μM nitrate and phosphate, respectively) while elevated nutrients refer to additions of 50 μM and 3 μM nitrate and phosphate, respectively

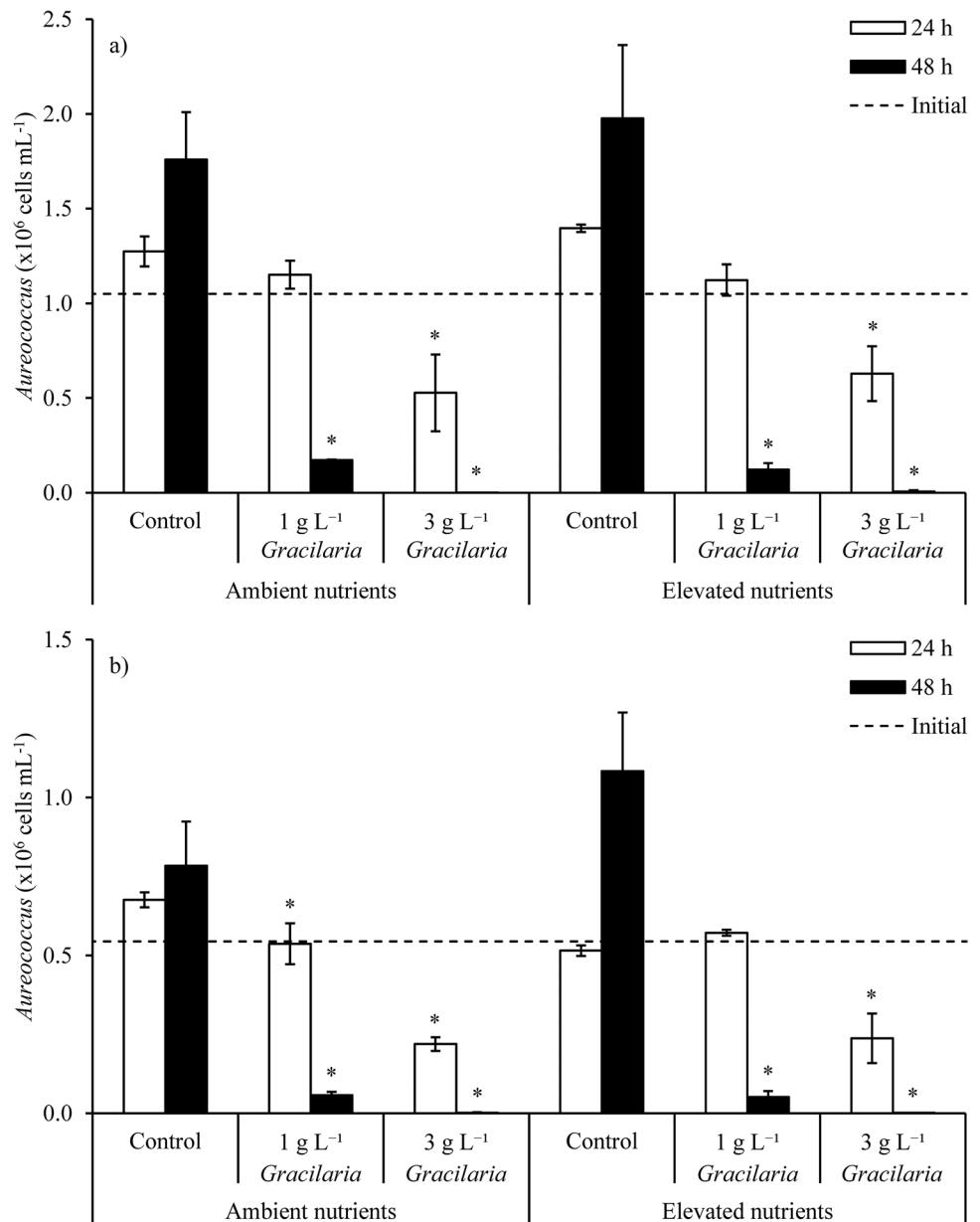
Figure #	Species	<i>A. anophagefferens</i> (cells mL^{-1})	<i>A. anophagefferens</i> strain	Macroalgal density (g L^{-1})	Experimental conditions
1	<i>G. tikvahiae</i>	1,000,000 and 500,000	1850	0.00, 0.25, 0.50, 0.75, 1.00	<i>A. anophagefferens</i> were exposed to <i>G. tikvahiae</i> , ambient and elevated nutrients
2	<i>G. tikvahiae</i>	1,000,000 and 500,000	1850	0.00, 0.25, 0.50, 0.75, 1.00	Least effective dose of <i>G. tikvahiae</i> ; elevated nutrients
3	<i>G. tikvahiae</i>	1,000,000 and 500,000	1850	0.00, 0.25, 0.50, 0.75, 1.00	Least effective dose of <i>G. tikvahiae</i> ; ambient nutrients
4	<i>D. japonica</i>	1,000,000 and 500,000	1850	0.00, 1.00, 3.00	<i>A. anophagefferens</i> exposed to ambient or elevated nutrients and <i>G. tikvahiae</i>
5	<i>D. japonica</i>	1,000,000 and 500,000	1850	0.00, 0.25, 0.50, 0.75, 1.00	Least effective dose of <i>D. japonica</i> ; elevated nutrients
6	<i>D. japonica</i>	1,000,000 and 500,000	1850	0.00, 0.25, 0.50, 0.75, 1.00	Least effective dose of <i>D. japonica</i> ; ambient nutrients
7	<i>D. japonica</i>	1,000,000	1850	0.00, 1.00, 3.00	<i>A. anophagefferens</i> exposed to <i>D. japonica</i> with and without antibiotic additions
8	<i>D. japonica</i>	500,000	1850	0.00, 0.50, 1.00	Ambient nutrients or 50 μM additions of nitrate or urea
Supp. Fig. S2	<i>D. japonica</i> and <i>U. lactuca</i>	750,000	1850	0.00, 0.50, 1.00, 3.00	Elevated nutrient added daily and antibiotics added to all treatments
Supp. Fig. S3	None	300,000	1850	0.00	<i>A. anophagefferens</i> exposed to increasing pH levels (8.00, 8.25, 8.50, and 9.00)
9	<i>D. japonica</i> and <i>G. tikvahiae</i>	350,000 – 400,000	1707, 1850, 1984	1.00	Three strains of <i>A. anophagefferens</i> exposed to macroalgae; ambient nutrients
10	<i>D. japonica</i> and <i>G. tikvahiae</i>	50,000	Various	0.00, 1.00, 3.00	Ambient and elevated nutrients

Fig. 2A; Supplementary Table S6) while the 0.75 g L^{-1} was lower than the control at 96 h in the low *Aureococcus* treatment only (Tukey HSD; $p < 0.05$; Fig. 2B; Supplementary Table S6). In the experiment without nutrients added, only the 1.00 g L^{-1} *G. tikvahiae* treatment with low *A. anophagefferens* was significantly lower than the control after 72 h (Tukey HSD; $p < 0.05$; Fig. 3B; Supplementary Table S6).

Dasysiphonia japonica

The algicidal effects of *D. japonica* were stronger than *G. tikvahiae* (Figs. 5 – 7). Experiments were established with *A. anophagefferens* at two densities (high and low, 1×10^6 and 5×10^5 cells mL^{-1} , respectively) that were exposed to three levels of *D. japonica* (0, 1, and 3 g L^{-1}) and two levels of nutrients (ambient and elevated; Fig. 4; Table 1). In the experiment with high levels of *A. anophagefferens*, dose of *D. japonica*, level of nutrients and the interaction between factors were all significant (Two-way ANOVA; $p < 0.001$ for

Fig. 1 a) high (1×10^6 cells mL^{-1}) and b) low (5×10^5 cells mL^{-1}) cell concentrations of *A. anophagefferens* exposed to ambient ($< 5 \mu\text{M}$ and $< 1 \mu\text{M}$ nitrate and phosphate, respectively) and elevated ($50 \mu\text{M}$ and $3 \mu\text{M}$ nitrate and phosphate, respectively) nutrient levels and increasing levels of *G. tikvahiae* (0, 1 and 3 g L^{-1}). Starting cell concentrations indicated by horizontal dashed line. Asterisks above bars denote significant results

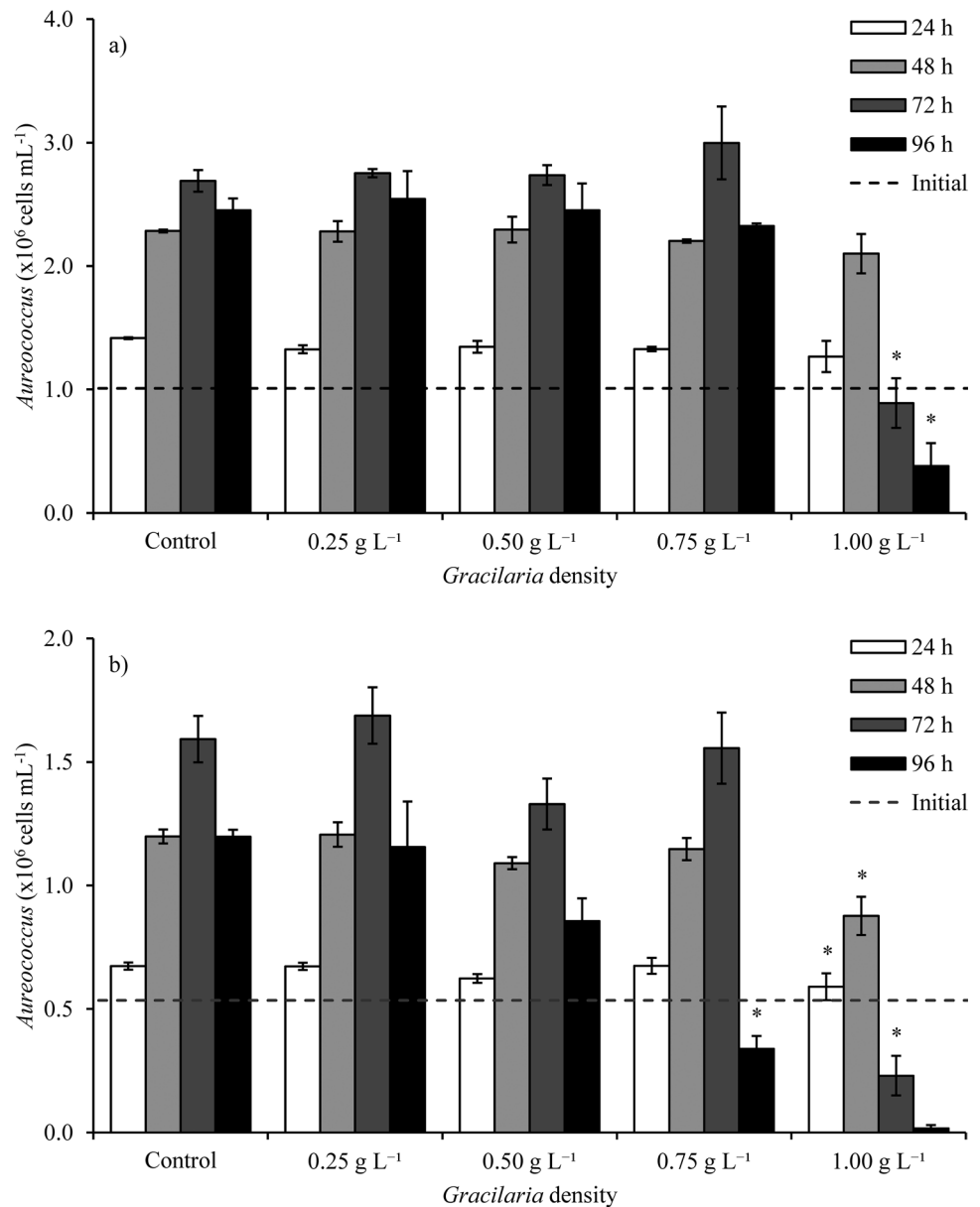


all; Fig. 4A; Supplementary Table S10). At low starting *A. anophagefferens* cell concentrations (Fig. 4B), dose of *D. japonica* was a significant factor, however, nutrients and the interaction were not (Two-way ANOVA; $p > 0.05$; Fig. 4B; Supplementary Table S11). *A. anophagefferens* cell concentrations were significantly lower than the control in the 1 g L^{-1} and 3 g L^{-1} treatments at both nutrient levels and both *A. anophagefferens* levels after 24 h (Tukey HSD; $p < 0.05$; Fig. 4; Supplementary Table S12). At the high *A. anophagefferens* level, cells concentrations in the 3 g L^{-1} treatment were significantly lower than at 1 g L^{-1} at each time points (Tukey HSD; $p < 0.05$; Fig. 4A; Supplementary Table S12) whereas at the lower starting *A. anophagefferens* level, cells densities in the 3 g L^{-1} treatment were significantly lower

than at 1 g L^{-1} at 24 and 48 h only (Tukey HSD; $p < 0.05$; Fig. 4B; Supplementary Table S12) as cells densities were reduced to $< 1,000 \text{ cells mL}^{-1}$ at both levels of *D. japonica* after 72 h regardless of nutrient level (Tukey HSD; $p < 0.05$; Fig. 4B; Supplementary Table S12).

In the next two experiments, *A. anophagefferens* at high and low cell concentrations (1×10^6 and $5 \times 10^5 \text{ mL}^{-1}$, respectively) were exposed to lower levels of *D. japonica*: 0.00 g L^{-1} (control), 0.25 , 0.50 , 0.75 , and 1.00 g L^{-1} , with and without nutrients supplementation (Table 1). Dose of *D. japonica* was a significant factor at both initial *A. anophagefferens* cell concentrations (One-way ANOVA; $p < 0.001$; Fig. 5; Supplementary Tables S13 and S14). In the experiment with high levels of *A. anophagefferens* with nutrients

Fig. 2 a) high (1×10^6 cells mL^{-1}) and b) low (5×10^5 cells mL^{-1}) cell concentrations of *A. anophagefferens* exposed to increasing densities of *G. tikvahiae* (0.00, 0.25, 0.50 and 1.00 g L^{-1}) with elevated nutrients (50 μM and 3 μM nitrate and phosphate, respectively). Starting cell concentrations indicated by horizontal dashed line. Asterisks above bars denote significant results

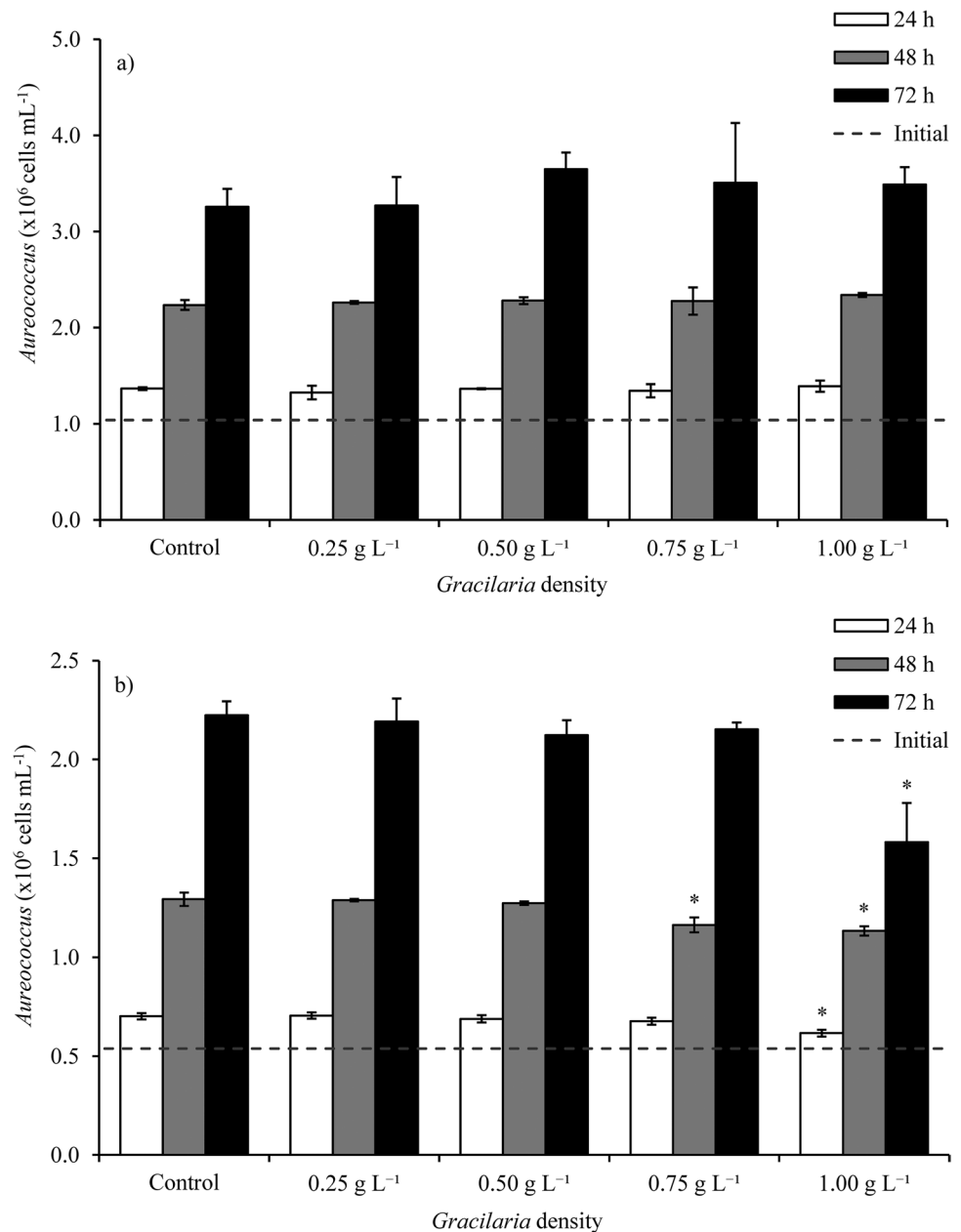


(Fig. 5A), there were no significant differences between any treatments after 48 h. After 72 h, however, in the higher initial *A. anophagefferens* level, cell concentrations were significantly lower than the control at the higher doses of *D. japonica* (0.75 and 1.00 g L^{-1}) (Tukey HSD; $p < 0.05$; Fig. 5A; Supplementary Table S15). In the low *A. anophagefferens* treatments, only the 1.00 g L^{-1} treatment had significantly lower concentrations than the control after 24 h, whereas all treatments that received *D. japonica* had significantly lower concentrations than the control after 48 h (Tukey HSD; $p < 0.05$; Fig. 5B; Supplementary Table S15). *A. anophagefferens* cell concentrations in the 0.25 g L^{-1} treatment were significantly higher than in the 0.75 and 1.00 g L^{-1} treatments (Tukey HSD; $p < 0.05$; Fig. 5B; Supplementary Table S15) but were not different than in

the 0.50 g L^{-1} treatment (Tukey HSD; $p > 0.05$; Fig. 5B; Supplementary Table S15). In addition, higher doses of *D. japonica* (0.75 and 1.00 g L^{-1}) resulted in significantly lower *A. anophagefferens* cell concentrations than the lower doses of *D. japonica* (0.25 and 0.50 g L^{-1}) after 72 h, regardless of starting *A. anophagefferens* cell concentrations (Tukey HSD; $p < 0.05$; Fig. 5; Supplementary Table S15).

The outcome of the experiment where *A. anophagefferens* at two densities (high and low, 1×10^6 cells mL^{-1} and 5×10^5 cells mL^{-1} , respectively) was exposed to increasing levels of *D. japonica* (0.00 – 1.00 g L^{-1}) without nutrients (Fig. 6) was very similar to the experiment when nutrients were present (Fig. 5). Dose of *D. japonica* was significant at both starting *A. anophagefferens* cell concentrations (One-way ANOVA; $p < 0.05$; Fig. 6; Supplementary Tables S16 and

Fig. 3 a) high (1×10^6 cells mL^{-1}) and b) low (5×10^5 cells mL^{-1}) cell concentrations of *A. anophagefferens* exposed to increasing densities of *G. tikvahiae* (0.00, 0.25, 0.50 and 1.00 g L^{-1}) with ambient nutrients ($< 5 \mu\text{M}$ and $< 1 \mu\text{M}$ nitrate and phosphate, respectively). Starting cell concentrations indicated by horizontal dashed line. Asterisks above bars denote significant results

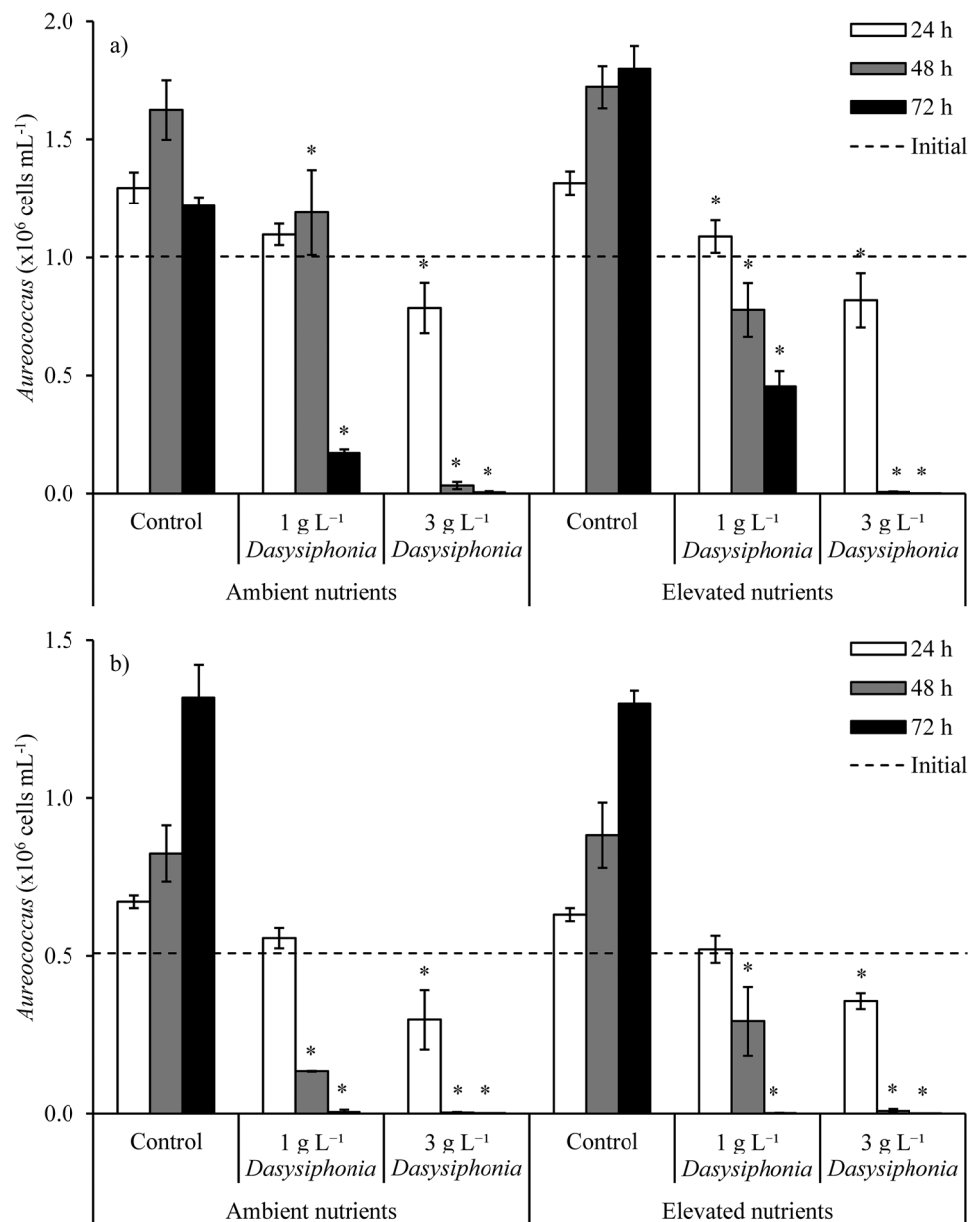


S17). In the high *A. anophagefferens* experiment, significant differences among levels of the rhodophyte emerged only at 72 h when all treatment levels except 0.25 g L^{-1} were lower than the control (Tukey HSD; $p < 0.05$; Fig. 6A; Supplementary Table S18). For the low *A. anophagefferens* treatments, after 72 h, cell concentrations in the 1.00 g L^{-1} level were lower than all other levels of *D. japonica* including the control (Tukey HSD; $p < 0.05$; Fig. 6B; Supplementary Table S18).

Effects of antibiotics

In experiments where *A. anophagefferens* was exposed to three levels of *D. japonica* (0, 1, and 3 g L^{-1}) with and without antibiotics added (Table 1), *D. japonica* dose, the presence of antibiotics and the interaction between factors were all significant (Two-way ANOVA; $p < 0.005$ for all; Fig. 7; Supplementary Table S19). After 24 h, *A. anophagefferens* cell concentrations were reduced to levels lower than the control in all treatments (Tukey HSD; $p < 0.05$; Fig. 7; Supplementary Table S20). Several treatments within the antibiotic treatment had cell concentrations significantly higher than the no antibiotic treatment. Within the 3 g L^{-1}

Fig. 4 a) high (1×10^6 cells mL^{-1}) and b) low (5×10^5 cells mL^{-1}) cell concentrations of *A. anophagefferens* exposed to ambient ($< 5 \mu\text{M}$ and $< 1 \mu\text{M}$ nitrate and phosphate, respectively) and elevated ($50 \mu\text{M}$ and $3 \mu\text{M}$ nitrate and phosphate, respectively) nutrient levels and increasing densities of *D. japonica* (0, 1, and 3 g L^{-1}). Starting cell concentrations indicated by horizontal dashed line. Asterisks above bars denote significant results

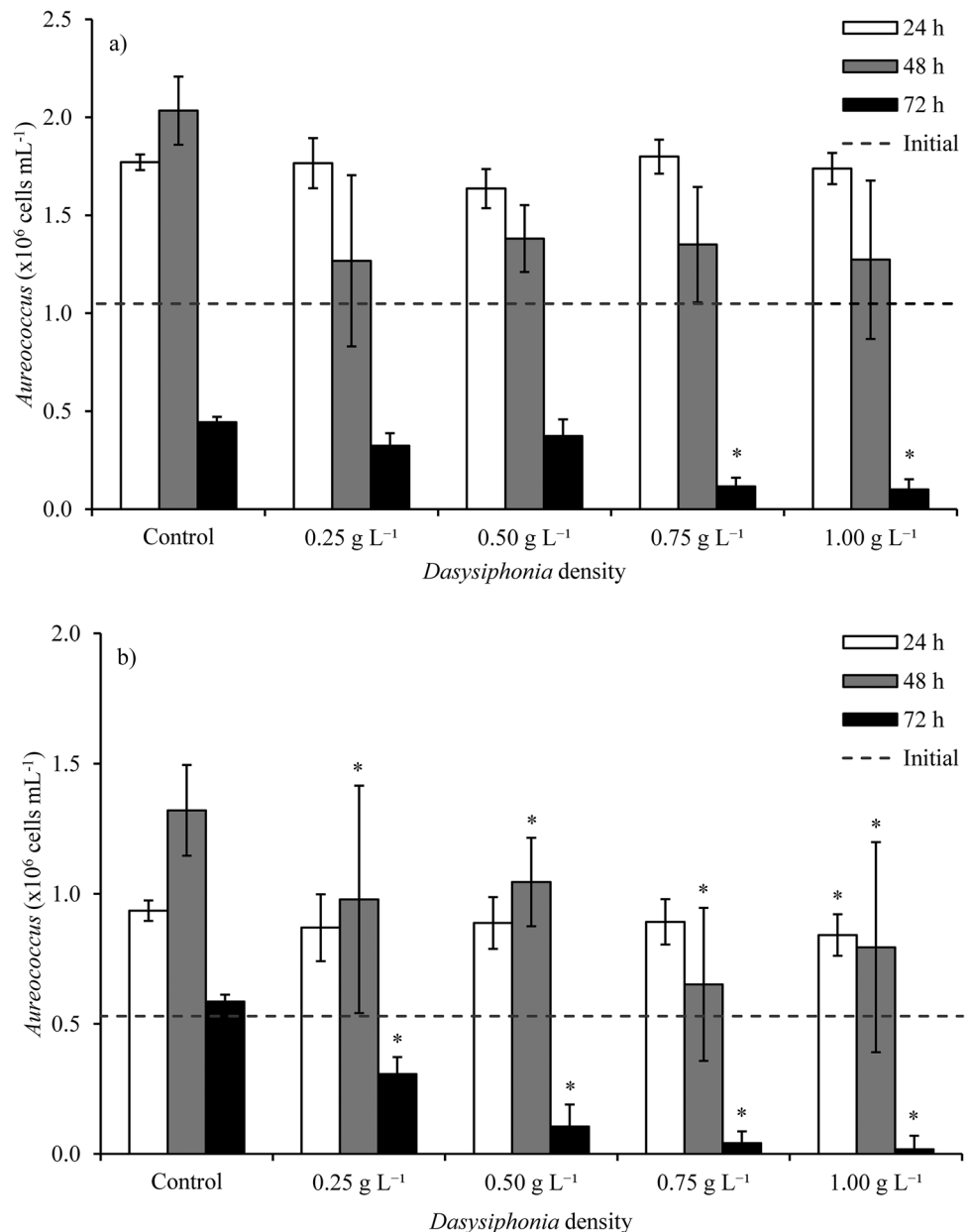


of *D. japonica* treatments, the no antibiotic treatment had significantly lower cell concentrations than the antibiotic treatment (Tukey HSD; $p < 0.05$; Fig. 7; Supplementary Table S20). After 72 h, however, *A. anophagefferens* was reduced to $< 1,000$ cells mL^{-1} in all treatments while control cell concentrations with and without antibiotics were more than 3×10^6 cells mL^{-1} (Tukey HSD; $p < 0.05$; Fig. 7; Supplementary Table S20). At 72 and 96 h, all 1 and 3 g L^{-1} seaweed treatment groups had significantly lower cell concentrations than either control treatment, but were not significantly different than each other, regardless of antibiotic additions (Tukey HSD; $p < 0.05$; Fig. 7; Supplementary Table S20).

Effect of nitrogen source

In an experiment exposing *A. anophagefferens* cultures grown on nitrate or urea to 0.0, 0.5 g L^{-1} , or 1.0 g L^{-1} of *D. japonica*, *D. japonica* level was a significant factor (Two-way ANOVA; $p < 0.001$; Fig. 8; Supplementary Table S21), while nutrient source and the interaction between factors were not (Two-way ANOVA; $p > 0.05$; Fig. 8; Supplementary Table S21). Still, there were differences in responses to *D. japonica* within nitrate-grown treatments compared to within urea-grown treatments at specific time points. For example, after 48 h, cell concentrations in the nitrate treatments were not significantly different from the control whereas in the urea

Fig. 5 a) high (1×10^6 cells mL^{-1}) and b) low (5×10^5 cells mL^{-1}) cell concentrations of *A. anophagefferens* exposed to increasing densities of *D. japonica* (0.00, 0.25, 0.50 and 1.00 g L^{-1}) with elevated nutrients (50 μM and 3 μM nitrate and phosphate, respectively). Starting cell concentrations indicated by horizontal dashed line. Asterisks above bars denote significant results



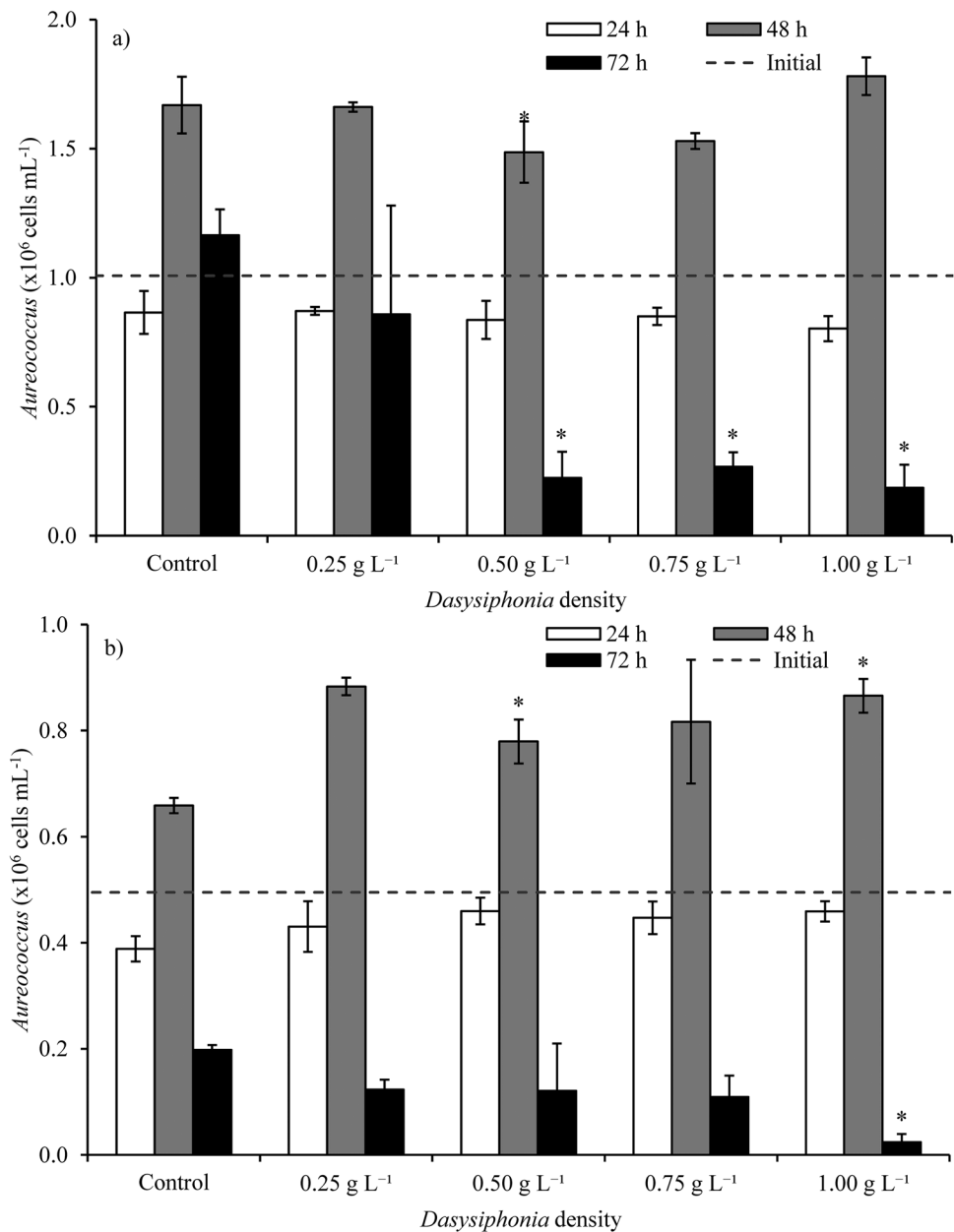
treatment, densities were significantly lower in the 1.0 g L^{-1} *D. japonica* treatment than in the control (Tukey HSD; $p < 0.05$; Fig. 8; Supplementary Table S22). At 72 and 96 h, cell concentrations in the 0.5 g L^{-1} and 1.0 g L^{-1} *D. japonica* treatments were significantly lower than the control in both nitrogen sources (Tukey HSD; $p < 0.05$; Fig. 8; Supplementary Table S22). After 96 h, cell concentrations in the urea grown 1.0 g L^{-1} *D. japonica* treatment were fourfold and significantly lower (1.1×10^5 cells mL^{-1}) than the nitrate treatment (4.0×10^5 cells mL^{-1} ; Tukey HSD; $p < 0.05$; Fig. 8; Supplementary Table S22). Also at 96 h, *A. anophagefferens* cell concentrations in both control groups were not significantly different than each other but were significantly higher than all treatments

exposed to nitrate (Tukey HSD; $p < 0.05$; Fig. 8; Supplementary Table S22). Moreover, *A. anophagefferens* cell concentrations in the 1.0 g L^{-1} treatments with urea were significantly lower than in the 0.5 g L^{-1} treatments with urea, but groups exposed to urea were not significantly different than the treatments exposed to nitrate, regardless of macroalgal density, at 96 h (Tukey HSD; $p < 0.05$; Fig. 8; Supplementary Table S22).

Comparing *Ulva* spp. to *D. japonica*

When *A. anophagefferens* was exposed to 0.0, 0.5, 1.0 and 3.0 g L^{-1} of *D. japonica* and *Ulva* spp., seaweed species was a significant factor (Two-way ANOVA; $p < 0.001$;

Fig. 6 a) high (1×10^6 cells mL^{-1}) and b) low (5×10^5 cells mL^{-1}) cell concentrations of *A. anophagefferens* exposed to increasing densities of *D. japonica* (0.00, 0.25, 0.50, and 1.00 g L^{-1}) with ambient nutrients ($< 5 \text{ } \mu\text{M}$ and $< 1 \text{ } \mu\text{M}$ nitrate and phosphate, respectively). Starting cell concentrations indicated by horizontal dashed line. Asterisks above bars denote significant results



Supplementary Fig. S2; Supplementary Table S23), but there was no significant interaction between seaweed concentration and species (Two-way ANOVA; $p > 0.05$; Supplementary Fig. S2; Supplementary Table S23). After 48 h, *A. anophagefferens* concentrations within the 3.0 g L^{-1} *D. japonica* and *Ulva* spp. treatments were significantly lower than the control treatments (Tukey HSD; $p < 0.05$; Supplementary Fig. S2; Supplementary Table S24) whereas at 72 h, *A. anophagefferens* concentrations were significantly lower than the control within the 1.0 and 3.0 g L^{-1} treatments of both macroalgae

(Tukey HSD; $p < 0.05$; Supplementary Fig. S2; Supplementary Table S24). By 96 h, cell concentrations were significantly lower in all treatments compared to the control (Tukey HSD; $p < 0.05$; Supplementary Fig. S2; Supplementary Table S24) and were significantly lower in the 0.5 g L^{-1} and 1.0 g L^{-1} treatment with *D. japonica* compared to the 0.5 g L^{-1} and 1.0 g L^{-1} treatments with *Ulva* spp. (Tukey HSD; $p < 0.05$; Supplementary Fig. S2; Supplementary Table S24).

Fig. 7 Cell concentrations of *A. anophagefferens* exposed to increasing densities of *D. japonica* (0, 1, and 3 g L⁻¹), with and without additions of antibiotics. Starting cell concentrations indicated by horizontal dashed line. Asterisks above bars denote significant results

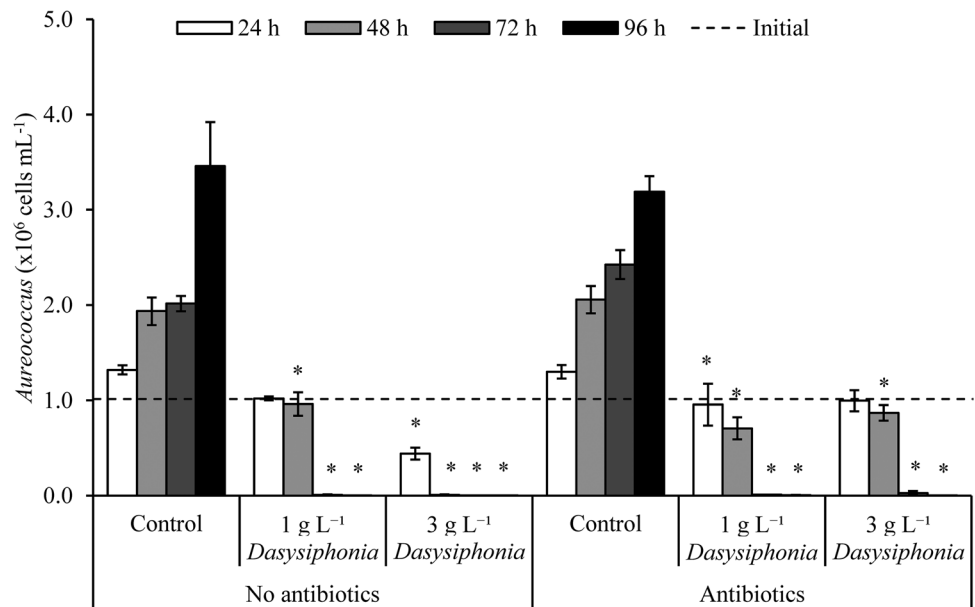
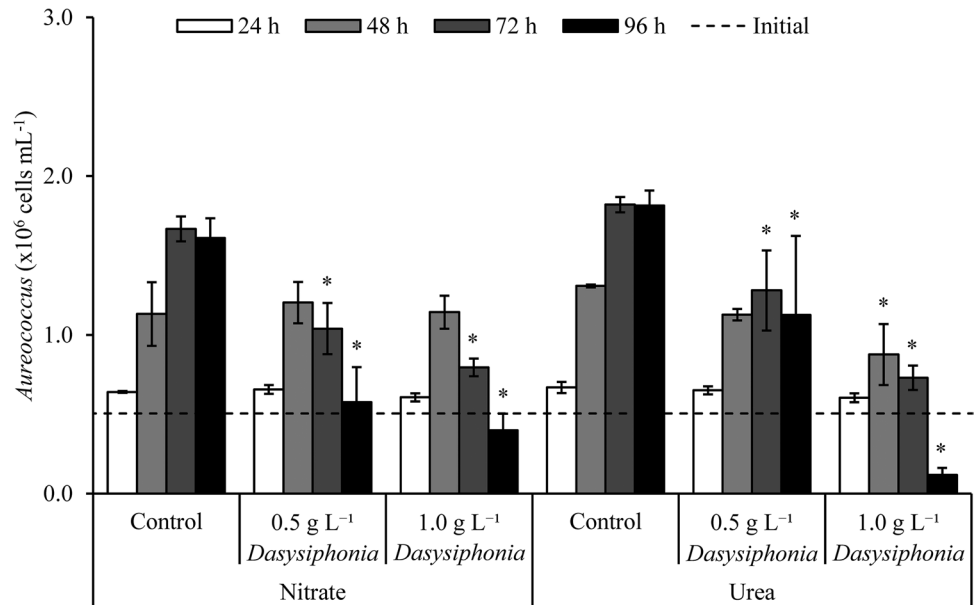


Fig. 8 Cell concentrations of *A. anophagefferens* when exposed to increasing densities of *D. japonica* (0.0, 0.5 and 1.0 g L⁻¹) and grown with nitrate or urea additions (50 μM for both). Starting cell concentrations indicated by horizontal dashed line. Asterisks above bars denote significant results



Effect of high pH on the growth of *A. anophagefferens*

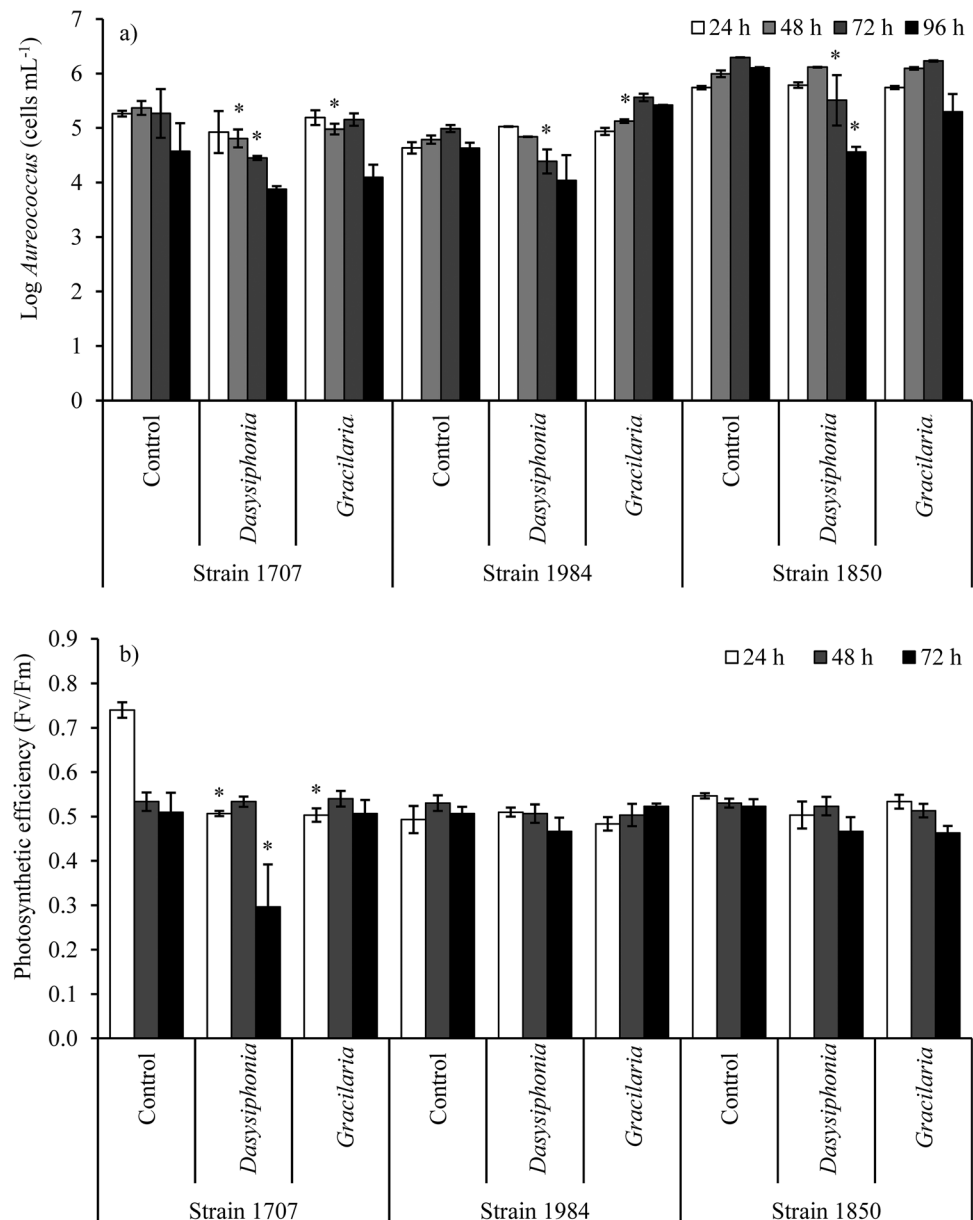
When *A. anophagefferens* was grown at a pH of 8.00, 8.25, 8.50 and 9.00, pH level was a significant factor for cell concentration (One-way ANOVA; $p < 0.05$; Supplementary Fig. S3; Supplementary Table S25). At 24 and 48 h, *A. anophagefferens* cell concentrations in the pH 9.00 treatment were significantly lower than all other treatments (Tukey HSD; $p < 0.05$; Supplementary Fig. S3; Supplementary Table S26). In contrast, cell concentrations were not significantly different across the pH 8.00, 8.25 and 8.50 treatments

(Tukey HSD; $p > 0.05$ for all; Supplementary Fig. S3; Supplementary S26).

A. anophagefferens multi-strain experiments

When strains CCMP1707, CCMP1984 and CCMP1850 were exposed to 1 g L⁻¹ of *D. japonica* and *G. tikvahiae*, macroalgal species was a significant factor (One-way ANOVA; $p < 0.05$ for all; Fig. 9A; Supplementary Table S27). For strain CCMP1707, after 48 h, *A. anophagefferens* concentrations within the *G. tikvahiae* and *D. japonica* treatments were significantly lower than the control treatment but were not significantly different than each other (Tukey

Fig. 9 a) cell concentrations and b) photosynthetic efficiency of three strains of *A. anophagefferens* exposed to 0 (control) and 1 g L⁻¹ of *D. japonica* and *G. tikvahiae*. Asterisks above bars denote significant results



HSD; $p < 0.05$; Fig. 9A; Supplementary Table S28). At 72 h, *A. anophagefferens* concentrations within the *D. japonica* treatments were significantly lower than the control and the *G. tikvahiae* treatment (Tukey HSD; $p < 0.05$; Fig. 9A; Supplementary Table S28). For strain CCMP1984, after 24 h, *A. anophagefferens* concentrations within the *G. tikvahiae* and *D. japonica* treatments were significantly lower than the control treatments (Tukey HSD; $p < 0.05$; Fig. 9A; Supplementary Table S28) but were not significantly different than each other (Tukey HSD; $p > 0.05$; Fig. 9A; Supplementary Table S28). After 48 h, *A. anophagefferens* concentrations within the *G. tikvahiae* treatments were significantly lower than the control treatments but significantly higher than the *D. japonica* treatments which were significantly lower than

the control (Tukey HSD; $p < 0.05$; Fig. 9A; Supplementary Table S28). By 72 h, *A. anophagefferens* concentrations within the *D. japonica* treatments were significantly lower than the control but concentrations in the *G. tikvahiae* treatments were not (Tukey HSD; $p < 0.05$; Fig. 9A; Supplementary Table S28). For strain CCMP1850, after 96 h, both *D. japonica* and *G. tikvahiae* treatments were significantly lower than the control, and *D. japonica* treatments were significantly lower than the *G. tikvahiae* treatments (Tukey HSD; $p < 0.05$; Fig. 9A; Supplementary Table S28).

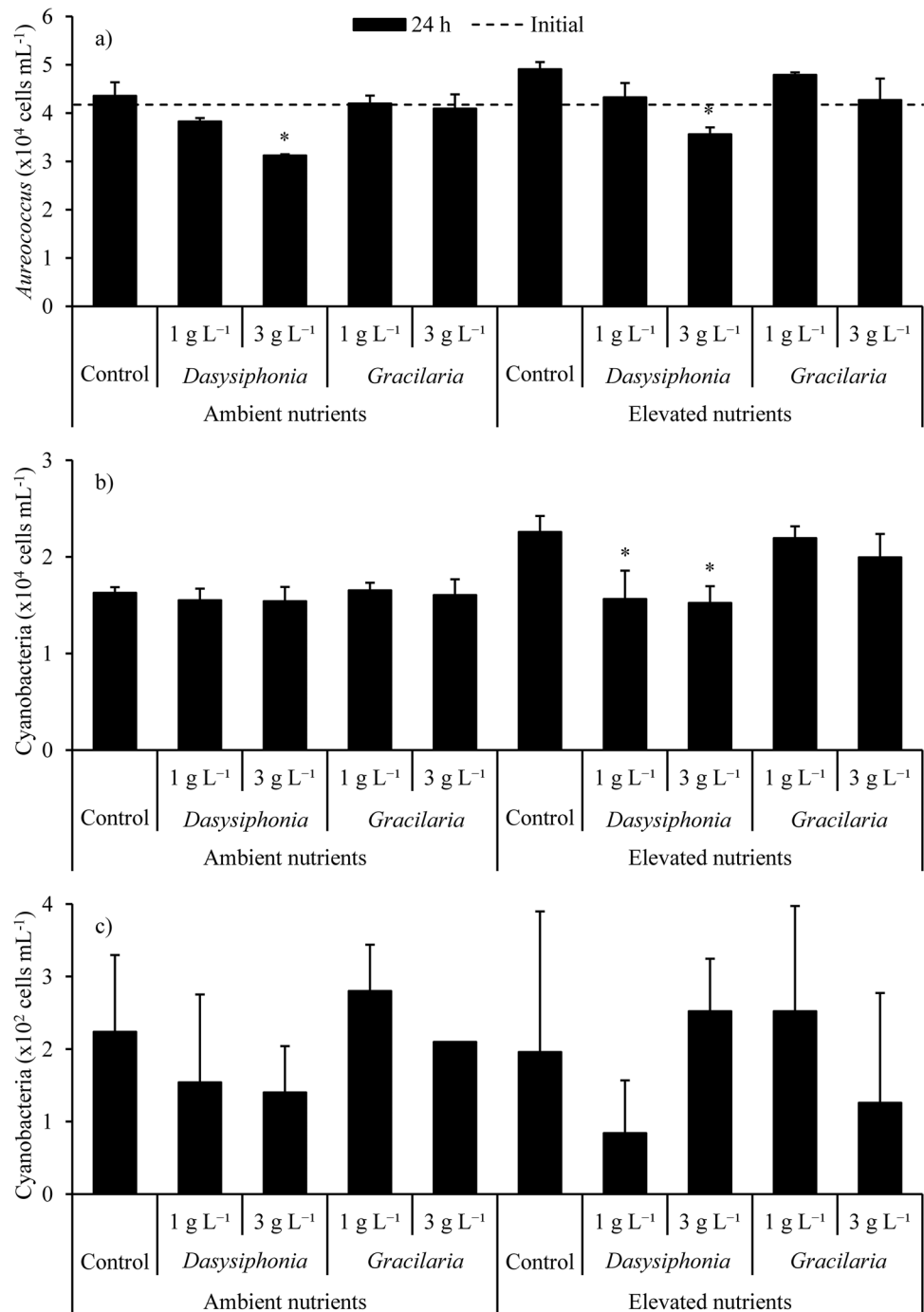
When the photosynthetic efficiency of *A. anophagefferens* was measured, macroalgal species was a significant factor for all strains (One-way ANOVA; $p < 0.05$ for all; Fig. 9B; Supplementary Table S29). For strain 1707, after

72 h, photosynthetic efficiency was significantly lower in the *D. japonica* treatment compared to the control and the *G. tikvahiae* treatment (Tukey HSD; $p < 0.05$; Fig. 9B; Supplementary Table S30). For strain 1984, photosynthetic efficiency was lower in the macroalgae treatments, but not significantly so (Tukey HSD; $p > 0.05$; Fig. 9B; Supplementary Table S30). For strain 1850, after 72 h, photosynthetic efficiency was significantly lower in both macroalgal treatments compared to the control (Tukey HSD; $p < 0.05$; Fig. 9B; Supplementary Table S30).

Great South Bay brown tide experiments

Finally, in the field experiment exposing bloom water collected from a brown tide event ($\sim 4.5 \times 10^4$ cells mL^{-1}) in Great South Bay to increasing levels of *D. japonica* and *G. tikvahiae* (0, 1, and 3 g L^{-1}) with and without nutrients added, species of seaweed, dose of seaweed and presence of nutrients were all significant factors, and there was a significant interaction between seaweed density and species (Three-way ANOVA; $p < 0.05$ for all; Fig. 10A;

Fig. 10 Cell concentrations of a) *A. anophagefferens* (5×10^4 cells mL^{-1}), b) phycocyanin-containing cyanobacteria and c) phycoerythrin-containing cyanobacteria from the Great South Bay after being exposed to increasing densities of *D. japonica* and *G. tikvahiae* (0, 1 and 3 g L^{-1}) for 24 h with and without nutrient additions. Starting cell concentrations indicated by horizontal dashed line. Asterisks above bars denote significant results



Supplementary Table S31). Within the ambient and elevated nutrient treatments, the treatment with 3 g L⁻¹ *D. japonica* had significantly lower *A. anophagefferens* concentrations (by 30%) than in the control and all other treatments (Tukey HSD; $p < 0.05$; Fig. 10A; Supplementary Table S32). In contrast, *G. tikvahiae* did not significantly reduce cell concentrations of *A. anophagefferens*.

Beyond *A. anophagefferens*, within treatments that received elevated nutrients, the 1 and 3 g L⁻¹ *D. japonica* treatments significantly reduced phycoerythrin-containing cyanobacteria concentrations relative to the control (Two-way ANOVA and Tukey HSD; $p < 0.05$ for all; Fig. 10B; Supplementary Tables S33 and S34) while the 1 and 3 g L⁻¹ *G. tikvahiae* treatment did not (Two-way ANOVA and Tukey HSD; $p > 0.05$ for both; Fig. 10B; Supplementary Tables S33 and S34). Within the ambient nutrient treatments, there were no significant differences in phycoerythrin-containing cyanobacteria concentrations between the control or the 1 and 3 g L⁻¹ *G. tikvahiae* treatments or between the seaweed treatments and the controls (Two-way ANOVA and Tukey HSD; $p > 0.05$ for all; Fig. 10B; Supplementary Tables S33 and S34). In addition, phycocyanin-containing cyanobacteria concentrations were not significantly different across any of the treatments (Two-way ANOVA and Tukey HSD; $p > 0.05$ for all; Fig. 10C; Supplementary Tables S35 and S36).

Discussion

In this study, *G. tikvahiae* and *D. japonica* significantly reduced cell concentrations of cultured and wild *A. anophagefferens* in a dose-dependent manner, with the effectiveness of each seaweed varying with initial *A. anophagefferens* cell concentration, nutrient levels, nitrogen source, seaweed density and the presence of antibiotics. Significant population reduction and growth inhibition caused by the rhodophytes was demonstrated across three clonal strains as well as a wild population of *A. anophagefferens*. The results suggest allelochemicals as a primary mechanism of anti-*A. anophagefferens* activity, though pH elevation, nutrient competition and seaweed-attached algicidal bacteria cannot be completely ruled out as secondary factors. Collectively, these results provide novel insight on the ecological role of native macroalgal species such as *G. tikvahiae* as well as non-native species, such as *D. japonica*, in the occurrence of HABs as well as the effectiveness of seaweed cultivation as a HAB mitigation approach.

Identifying the lowest minimum dose needed to inhibit *A. anophagefferens* is important for scaling experimental results to an aquaculture and/or ecosystem setting (Sylvers and Gobler 2021). Experiments measuring the lowest effective dose of *G. tikvahiae* and *D. japonica* indicated

that when *A. anophagefferens* concentrations were low and nutrients were elevated, relatively low levels of *D. japonica* (0.25 g L⁻¹) and *G. tikvahiae* (0.50 g L⁻¹) were sufficient to significantly reduce cell concentrations. At higher *A. anophagefferens* concentrations, however, the phytoplankton was more resistant, with effects manifesting later (≥ 72 h) and only with higher levels of seaweed (≥ 0.75 g L⁻¹). When nutrients were absent, *A. anophagefferens* was more resistant to the effects of the seaweed, with 0.50 g L⁻¹ *D. japonica* or 1.00 g L⁻¹ *G. tikvahiae* needed to reduce cell densities at 72 h. At lower initial *A. anophagefferens* concentrations and with low nutrients, *A. anophagefferens* was most resistant to *G. tikvahiae* and *D. japonica* with 1.00 g L⁻¹ of both species required to reduce cell concentrations after 72 h. It is worth noting here that, while 1.00 g L⁻¹ of branching rhodophytes is unlikely to occur in a natural ecosystem setting, it is a very modest concentration for shallow-water in-situ seaweed aquaculture installments, which can reach > 4 g L⁻¹ at low tide (Sylvers & Gobler 2021). These findings emphasize that the precise dose dependency of *A. anophagefferens* inhibition by rhodophytes is dependent upon nutrient status as well as initial *A. anophagefferens* populations size cell concentrations, with *A. anophagefferens* seemingly more resistant to *G. tikvahiae* and *D. japonica* at lower *A. anophagefferens* concentrations and low nutrients. These findings suggest that brown tides may be more sensitive to rhodophytes at bloom peak when cell concentrations are higher, and within near-shore regions where nutrient levels are higher than more open-water estuarine regions (SCDHS 1990–2020). This suggests that natural branching rhodophyte populations are unlikely to prevent the initiation of brown tide events but may play a role in limiting maximal *A. anophagefferens* cell concentration in such blooms, especially in more eutrophic estuaries.

We obtained multiple lines of evidence demonstrating that the growth inhibiting effects of *D. japonica* were significantly stronger than *G. tikvahiae*. Within the ambient and elevated nutrient treatments, *A. anophagefferens* cell concentrations were lower in *D. japonica* treatment groups than all equivalent *G. tikvahiae* treatments when compared across equivalent seaweed densities. Moreover, *A. anophagefferens* cell concentrations were significantly lower in the *D. japonica* treatment group than in all *G. tikvahiae* treatments by 72 h for strain CCMP1707 and by 96 h for strains CCMP1984 and CCMP1850. Finally, in the field experiment, cell concentrations of wild populations of *A. anophagefferens* were significantly reduced by 30% in 24 h by *D. japonica* but were unaffected by *G. tikvahiae*. The higher potency of *D. japonica* was somewhat unexpected given that *Gracilaria* spp. are known for their growth inhibiting impacts on HABs (Ye and Zhang 2013; Ye et al. 2014; Yang et al. 2015). During the field experiment, the inhibition of the growth of phycoerythrin-containing cyanobacteria by *D.*

japonica when nutrients were elevated, but not *G. tikvahiae*, suggests that *G. tikvahiae* may inhibit phytoplankton more specifically while *D. japonica* may inhibit more, but only when there is an adequate supply of nutrients.

Beyond *G. tikvahiae*, *D. japonica* also had stronger growth inhibiting effects than *Ulva* spp., an algal that is perhaps the best known for inhibiting the growth of many HABs (Jin and Dong 2003; Nan et al. 2004; 2008; Jin et al. 2005; Sylvers & Gobler 2021) including *A. anophagefferens* (Tang and Gobler 2011). In the experiment comparing *D. japonica* and *Ulva* sp., cell concentrations were significantly lower in the *D. japonica* treatments than the *Ulva* spp. treatments. Given this and the ability of *D. japonica* to inhibit growth even at low concentrations (0.25 g L^{-1}), the anti-algal effects of *D. japonica* appear to be among the most potent documented to date.

Several mechanisms including nutrient competition by seaweeds, pH elevation by seaweed photosynthesis, and the growth of algicidal bacteria in seaweed biofilms could be responsible for the growth inhibiting effects of seaweeds on *A. anophagefferens* (Hansen 2002; Imai et al. 2006; Sylvers and Gobler 2021). Since *A. anophagefferens* populations are influenced by organic and inorganic nitrogen (Berg et al. 1997; Gobler et al. 2002), nutrient source may also influence seaweed-based anti-algal activity against *A. anophagefferens*. Regarding nutrient levels, Smith and Horne (1988) reported that *Ulva pertusa* competed for inorganic nitrogen to reduce the growth of phytoplankton while Huo et al. (2011) reported that *Gracilaria verrucosa* inhibited the survival and growth of the harmful phytoplankton *Karenia mikimotoi* via nutrient competition. During our study, inhibition of *A. anophagefferens* growth was partly dependent on nutrient source and concentration of added nutrients. Additionally, there were significant differences in the extent of inhibition, especially temporally. For example, cell concentrations were more inhibited when urea was used as a nitrogen source over nitrate, and this inhibition occurred sooner in the urea treatments than in the nitrate treatments, suggesting a possible interaction between allelopathic compounds and the urea cycle in *A. anophagefferens* (Allen et al. 2011; Gobler et al. 2011). Furthermore, cultures that were supplemented with nutrients experienced more severe declines in cell densities than nutrient free cultures during early stages (48–72 h) of exposure to *G. tikvahiae* but not *D. japonica*. Still, by the end of experiments with *G. tikvahiae*, *A. anophagefferens* cell concentrations were extremely low, regardless of nutrient levels. Therefore, nutrients had significant effect on the inhibition of *A. anophagefferens* by rhodophytes, though given that the levels of inhibition were similar by the end of experiments, it is likely a secondary effect.

Significantly increased pH in higher density seaweed treatments correlates with reduction in *A. anophagefferens*

cell density; however, causation is difficult to disentangle from this, as pH elevation from seaweed photosynthesis is expected in these experiments (Ye et al. 2014; Raven et al. 2020) and may have no bearing on observed *A. anophagefferens* levels (Supplementary Figs. S3–S15). High pH has been cited as a cause of phytoplankton impairment (Hansen 2002; Pedersen and Hansen 2003; Middelboe and Hansen 2007) and thus could have been the mechanism of *A. anophagefferens* decline in experiments. In the experiment exploring the effect of pH on *A. anophagefferens*, however, reduced *A. anophagefferens* cell concentrations were only observed within the highest pH treatment group (9.00), a level never observed in any macroalgae treatment during this study. Still, given that growth was unaffected at 8.50 and reduced at 9.00 and given that a pH between 8.50 and 9.00 occurred in some treatments, it is plausible that some of the growth reduction could have been associated with elevated pH. There were, however, many more instances of reduced *A. anophagefferens* in the presence of macroalgae at pH < 8.50, indicating that in this study, pH elevation was a very minor factor in the observed anti-*A. anophagefferens* effects of the seaweeds.

Beyond pH and nutrients, previous studies have also documented high levels of heterotrophic bacteria with algicidal properties growing in the biofilms of seaweeds (Imai et al. 2006) and some bacteria have been shown to cause *A. anophagefferens* growth inhibition (Frazier et al. 2007). During the experiment focusing on the effects of antibiotics on *A. anophagefferens* growth rates, there were minor effects from the antibiotics. For example, cultures grown with antibiotics experienced a 24 h delay in the reduction of cell concentrations at the highest macroalgae treatment. Still, these differences were not detected at the lower level of seaweed biomass, and after 72 h, *A. anophagefferens* populations in all treatments collapsed, regardless of whether antibiotics were administered. Hence, while seaweed-associated algicidal bacteria may have had a minor impact on *A. anophagefferens*, they were clearly not the primary growth-inhibiting mechanism, as the ultimate fate of seaweed-exposed *A. anophagefferens* was the same with or without added antibiotics.

Given that *G. tikvahiae* and *D. japonica* reduced *A. anophagefferens* cell abundance when nutrient concentrations were high or low, when cultures were grown on nitrate or urea, despite pH elevation being lower than levels observed to inhibit *A. anophagefferens*, and whether or not antibiotics were administered, allelochemicals were likely the primary anti-*A. anophagefferens* mechanism of the studied seaweeds. While this study did not measure the allelochemicals released by the macroalgae, results from prior studies have identified polyunsaturated fatty acids as the compounds responsible for algicidal allelochemicals in several species of red (Ye et al. 2014; Yang et al. 2015) and green seaweeds (Alamsjah et al. 2005; Tang and Gobler 2011). Furthermore,

Tang and Gobler (2011) demonstrated that the dried, powdered *U. lactuca* contained water-soluble and heat-stable allelochemicals responsible for inhibiting the growth microalgae. Similarly, Ye et al. (2013) demonstrated that allelopathic compounds of *Gracilaria lemaneiformis* could effectively inhibit photosynthesis of *Chaetoceros curvisetus* at a molecular level, by decreasing the number of active photosynthetic reaction centers, causing damage to the oxygen-evolving complex and blocking electron transport. In this study, when the photosynthetic efficiency of *A. anophagefferens* was measured, it was significantly reduced by *D. japonica* and to a lesser extent by *G. tikvahiae*, which could be evidence of the production of similar allelochemicals that impact photosystem II by these seaweeds (Suggett et al. 2003). Prior studies have also demonstrated that wounding of macroalgal tissue, such as the cutting during preparation phase of the current study, may trigger the release of potentially toxic compounds that could be allelopathic (Hammann et al. 2016; Sylvers and Gobler 2021). Although the identity of some seaweed-produced allelochemicals have been suggested, further research is needed to isolate potential allelochemicals in the red seaweeds studied here (*D. japonica* and *G. tikvahiae*) and to identify the allelopathic mechanisms specific to each of these species.

It is plausible that the temporal and spatial dynamics of the seaweeds studied here may influence the occurrence of brown tides cause by *A. anophagefferens*. This picoplankton achieves high densities within the open waters of estuaries, but its cell concentrations decline by orders of magnitudes in shallow tributaries where salinities are not significantly different from neighboring, open estuarine sites (SCDHS 1990–2020); a major difference between these environments is the presence of much higher densities of seaweeds in the tributaries (Tang and Gobler 2011). In addition, *D. japonica* has recently invaded the same south shore lagoons that host brown tides cause by *A. anophagefferens* (Young and Gobler 2021), and since this invasion has been documented (2018), brown tides in these lagoons have been less intense (10^5 cells mL⁻¹ vs. 10^6 cells mL⁻¹) and shorter in duration (weeks vs. months) compared to before the invasion (SCDHS 1990–2020). As such, it is possible that macroalgae influence the occurrence of brown tides in NY estuaries.

The observed anti-*A. anophagefferens* effects of seaweeds in this study, along with the general anti-algal effects of seaweeds shown in other studies, suggest that seaweed cultivation is a potentially viable technique for HAB control and mitigation. It is especially promising among HAB mitigation strategies in that it could be cost-neutral or even profit generating for marine farmers, as seaweeds are a viable commercial product. In conjunction with the growing evidence suggesting that rhodophytes are practical candidates for mitigating marine HABs (Ye et al. 2013, 2014; Yang et al. 2015), there are several,

broader facets of rhodophyte cultivation that make them an ideal option for seaweed aquaculture-based HAB mitigation strategies. When harvested, red seaweeds produce a higher yield compared to other seaweed phyla, and provide economically valuable extracts (FAO 2020). In some instances, rhodophytes, including *Gracilaria* spp., can be a key component of integrated multi-trophic aquaculture (IMTA) systems whereby fish and/or shellfish are co-cultured with rhodophytes such as *Porphyra purpurea*, and *G. lemaneiformis*, which are harvested for commercial use (Chopin et al. 2001; Neori 2007; Ye et al. 2014; Yang et al. 2015). In other instances, seaweeds have been purposely deployed to control nutrient levels within estuarine systems (Neori et al. 2004; Yang et al. 2006). In the future, regenerative ocean farms (aquaculture of bivalves and/or seaweeds that does not rely external inputs such as food for fish) and IMTA systems culturing *Gracilaria* spp. could have compounding benefits including the reduction of coastal anthropogenic nutrient loading and mitigation of HABs as well as providing commercially valuable products (Yang et al. 2015; Buschmann et al. 2017; Mixon 2017). When considering the cultivation of *G. tikvahiae*, molecular confirmation of the species is important, as *Agarophyton vermiculophyllum*—an invasive red seaweed that is morphologically indistinguishable from *G. tikvahiae*—has an overlapping range with *G. tikvahiae* and is thought to produce potentially toxic compounds (Nejrup et al. 2012; Nettleton et al. 2013). Despite the higher magnitude of anti-*A. anophagefferens* activity attributed to *Dasysiphonia japonica*, this species is invasive and potentially harmful to juvenile fish and shellfish (Young et al., in prep), making it unsuitable for purposeful cultivation.

In summary, this study demonstrated that exposure of the harmful pelagophyte, *Aureococcus anophagefferens* to the rhodophytes, *Gracilaria tikvahiae* and *Dasysiphonia japonica*, significantly, strongly, and quickly (24–96 h) reduces *A. anophagefferens* cell concentrations, and in many cases results in a complete collapse of *A. anophagefferens* populations. Given that changing levels of nutrients, pH, and bacterial levels had minor effects on *A. anophagefferens* and affected only the timelines rather than the magnitude of the observed population reductions, allelopathic compounds were suggested as the primary mechanism of anti-*A. anophagefferens* action. Our results suggest that the recent invasion of *D. japonica* in NY estuaries (Young and Gobler 2021), especially in Great South Bay, may be influencing the patterns of brown tide occurrence, potentially causing shorter and less intense blooms. Finally, the strategic deployment of *G. tikvahiae* as an element of open-water coastal aquaculture systems may be an environmentally friendly and revenue-generating option for preventing and/or controlling blooms caused by *A. anophagefferens* and likely other harmful algae.

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Data Availability Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Declarations

Competing Interests The authors declare that they have no competing interests to declare that are relevant to the content of this article.

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