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Decreased Irradiance and Nutrient Enrichment Mitigate the Negative Effect of Ocean Warming on Growth and Biochemical Compositions of a Canopy-Forming Marine Macroalga

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Abstract: Heatwaves under global warming have negative impacts on ecosystem primary producers. This warming effect may be synergized or antagonized by local environments such as light and nutrient availability. However, little is known about the interactive effects of warming, irradiance, and nutrients on physiology of marine macroalgae, which are dominant in coastal ecosystems. The present study examined the combined effects of warming (23 and 26 °C), irradiance (30 and 150 µmol photon m^{-2} s^{-1}), and nutrients (enriched and non-enriched) on specific growth rate (SGR) and biochemical compositions of the canopy-forming marine macroalga *Sargassum fusiforme*. The negative effect of warming on SGR and ratio of chlorophyll (Chl) *c* to Chl *a* was antagonized by decreased irradiance. Moreover, the negative effect of temperature elevation on carbon content was antagonized by nutrient enrichment. These results suggest that the effect of warming on the growth and carbon accumulation of this species can be mitigated by decreased irradiance and nutrient enrichment.

Keywords: carbon sequestration; climate change; ecosystem conservation; marine macroalgal forest; non-additive effect

1. Introduction

The recent global warming and associated climate change are thought to be attributable to an increase in the level of atmospheric CO_2, mainly caused by the burning of fossil fuels [1]. In order to mitigate climate change, the conservation and restoration of forests and vegetated coastal ecosystems are important because they effectively fix and sequester atmospheric CO_2 for a long time [2,3]. However, the increasing intensity, frequency, and duration of heatwaves occurring as a result of global warming have been negatively affecting the biomass and productivity of ecosystem primary producers, including terrestrial plants and marine macroalgae [4–7]. At the same time, increasing evidence suggested that the effect of this global stressor on primary producers can be synergized or antagonized by local nutrient environments [8–10]. Moreover, physiological studies have shown that heat stress combined with excess light energy causes photoinhibition (i.e., decline of photosystems II efficiency) through an increase in reactive oxygen species production in chloroplast [11,12]. The knowledge of such interactions among multiple stressors is significant for ecosystem conservation, because the improvement of local environment is much easier compared to the management of global climate [13,14].

Marine brown, red, and green macroalgae are the main primary producers in coastal ecosystems. Specifically, marine macroalgal forests, dominated by large brown algae such as kelp (Laminariales) and fucoid (Fucales), are highly productive and provide food, habitat,

nursery, and spawning grounds for various marine organisms [15,16]. Conservation of macroalgal forests is expected to contribute to the mitigation of climate change because these algae can store and sequester carbon more than they have been considered [3,17]. However, recent ocean warming caused regional declines of macroalgal forests worldwide [6]. This regional scale-dependency may partially be explained by the interactive effects of ocean warming and local nutrient environments on macroalgal growth, which were detected in both kelp [18,19] and fucoid species [20–24]. Moreover, the effect of warming on photoinhibition was only detected under relatively high irradiance combined with nutrient enrichment conditions in the kelp *Eisenia bicyclis* [25]. This implies that the combined effects of temperature and nutrient availability on macroalgal growth may also depend on irradiance conditions. However, the combined effects of temperature, nutrients, and irradiance on macroalgal growth have rarely been examined [18,26], especially in fucoid species.

Photosynthetic pigments, including chlorophyll (Chl) *a*, accessory Chl, and photoprotective xanthophyll, are commonly used as abiotic stress markers of plants [27]. For example, elevated irradiance often causes decreases in Chl *a* content and ratio of accessory Chl to Chl *a* [28,29], because excess light energy enhances the production of reactive oxygen species in chloroplasts, which causes chlorophyll degradation. Moreover, high light acclimation is known to cause an increase in xanthophyll cycle pigments, including violaxanthin, antheraxanthin, and zeaxanthin, which dissipate the excess light energy as heat to avoid photoinhibition, in terrestrial plants and brown algae [30]. The effect of strong irradiance on these pigments may be synergized by an elevated temperature, because excess light energy combined with warm conditions promotes the production of reactive oxygen species [11]. However, little is known about the combined effects of warming and other factors on macroalgal pigments.

The carbon and nitrogen contents of marine macroalgae are important variables because the former is used to estimate the amount of carbon fixation and the latter is associated with high-temperature tolerance [31]. Previous studies have shown that carbon contents in kelp and fucoid species were rarely affected by variations in temperature and nutrient availability [19,26,32–34]. Nitrogen contents in these taxa commonly increased through nutrient enrichment but were rarely affected by temperature variation [18,19,26,34–37], except for some studies, which showed a decrease or an increase in the value in response to elevated temperature [25,32]. On the other hand, our previous study found complex interactions among temperature, nutrient availability, and irradiance affecting carbon and nitrogen contents in the kelp *E. bicyclis* [18]. Hence, a further multifactorial approach is necessary to understand the abiotic effects on these contents.

Fucoid brown algae belonging to the genus *Sargassum* are one of the dominant taxa in temperate and tropical reefs. Most *Sargassum* species have perennial holdfasts and stipes, and annual shoots (i.e., main branches). These shoots dislodged from reefs often float on the sea surface for a long time [38,39] and provide habitats and spawning grounds for various organisms [40,41]. Among the *Sargassum* spp., *Sargassum fusiforme* is one of the most important species because it is edible and has been cultivated in Asian countries including China, Korea, and Japan [42]. Although several studies have already shown the effect of warming on the performance of this species [43,44], the combined effects of warming, irradiance, and nutrients have never been tested.

In the present study, the combined effects of warming, nutrient enrichment, and irradiance on specific growth rate (SGR) and biochemical compositions, including carbon, nitrogen, and pigment contents, were evaluated by a laboratory culture experiment. We addressed the following questions: (1) Is the effect of warming on SGR synergized or antagonized by nutrient enrichment and elevated irradiance? (2) Are there complex interactions among these three factors on biochemical compositions?

2. Materials and Methods

2.1. Preparation of Specimens

Six *S. fusiforme* individuals, which had more than nine shoots, were collected in January 2020 from a depth of 1–2 m along the Kitsunezaki coast (38°21′01″ N, 141°25′06″ E), Oshika Peninsula, Miyagi Prefecture, northeastern Japan. The average seawater temperature during summer between 2011 and 2017 was ca. 23 °C, while summer temperatures were 3.1 and 3.3 °C higher than the average in 2012 and 2015, respectively, at a depth of 0.8 m near the sampling site [45]. The ranges of irradiance, and ammonium, nitrate, and phosphorus concentrations were 2.0–21.8 mol photon m^{-2} d^{-1}, 0.22–3.76 μM, 0.22–1.64 μM, and 0.11–0.28 μM, respectively, during same periods (2011–2017) at the same depth [45].

The specimens were transported to the laboratory in insulated cool boxes, and were cleaned with sterile seawater to remove epiphytes and diatoms. The apical potions of the shoots, which include meristems, were excised into 3 cm fragments [43]. This procedure allowed the cultivation of these shoots using flasks. In order to offset the negative effects of excision, the shoots were put into several 1L flasks with artificial seawater (AW) without nitrate or phosphate (LIVESea, DELPHIS Co., Hyogo, Japan), and were placed in an incubator (FLI-2000A, Tokyo Rikakikai. Co., Ltd., Tokyo, Japan) at the optimal growth temperature of 20 °C (under nutrient limitation, [24]) and an irradiance of 70 μmol photon m^{-2} s^{-1} (as an intermediate level between two irradiance treatments in this study) with a photoperiod of 12 h L (light):12 h D (dark) for 2 days.

2.2. Experimental Design

These sampled shoots were cultured for 9 d in eight different treatments (one shoot per flask, six flasks per treatment). Six shoots derived from six different individuals were used as replicates. This culture experiment was a three-way factorial design ($2 \times 2 \times 2$ treatments), consisting of two temperature levels (23 and 26 °C), two nutrient levels (enriched and non-enriched), and two irradiance levels (30 and 150 μmol photon m^{-2} s^{-1}).

The temperature levels were chosen because the average and highest seawater temperature during summer were ca. 23 and 26 °C, respectively, in this region [18,45]. In addition, seawater temperatures are predicted to increase by ca. 3 °C by the end of the year 2100 [46]. The two irradiance levels were set based on the compensation and optimal growth irradiance of this species, which reportedly occurred at 5–37 [47] and 100–180 μmol photon m^{-2} s^{-1} [48], respectively.

The nutrient enriched and non-enriched treatments were prepared using 5% Provasoli's enriched seawater (PESI) [49] and AW (see Section 2.1), respectively. This is because the growth of *Sargassum* spp. increases in response to moderate nutrient enrichment but declines by excessive enrichment [50]. In contrast, the absence of any effect of nutrient enrichment using 25% PESI on the growth of *Sargassum patens* in our previous study [51] implied that nitrate concentration in 25% PESI (ca. 200 μM in dissolved inorganic nitrogen, [29]) might be too high for *Sargassum* species. Although 5% PESI theoretically includes 41.2 μM nitrate and 1.6 μM glycerophosphate, nitrate concentrations in these media were confirmed using a portable water analyzer (HS-1000SW, HUMAS-Co., Ltd., Jeonmin-Dong, Korea) with five replications. The culture media in each flask was changed every 3 d [18]. The AW salinity, measured using a salinity meter (Horiba LAQUA act, HORIBA Advanced Techno Co., Ltd., Kyoto, Japan), was 34 psu.

2.3. Measurements of SGR and Biochemical Compositions

The wet weights of the shoots before and after culturing (initial and final wet weight, respectively) were measured using an electronic balance (0.01 g accuracy) after removal of excess moisture by blotting on paper towel. An exponential growth was assumed and SGRs (% d^{-1}) were determined as 100 × ln (final wet weight/initial wet weight)/9 d. The shoots after culturing and the other six shoots before culturing were used to measure final and initial biochemical compositions, respectively. Carbon and nitrogen contents were measured using an organic elemental analyzer (FLASH2000, ThermoFisher Scientific,

Waltham, MA, USA) after the samples were placed in a dry oven (EYELA WFO-500, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) for 12 d at 80 °C. Pigment contents, including Chl *a*, Chl c_2, fucoxanthin (Fuco), violaxanthin, antheraxanthin, and zeaxanthin were measured using high-performance liquid chromatography (HPLC) after the samples were placed in 9 mL bottles containing 5 mL of dimethylformamide (DMF). The samples were diluted in distilled water to achieve 80% supernatant and were analyzed by an HPLC autosampler (Shimadzu, Japan) following similar methods by Zapata et al. [52]. The guard column was placed between the injection valve and the analytical column (Symmetry C8, Waters Milford, MA, USA). The sum of xanthophyll cycle pigments, including violaxanthin, antheraxanthin, and zeaxanthin contents (VAZ), was calculated. Only Chl *a* content and the ratio of other pigments (Chl c_2, Fuco, and VAZ) to Chl *a* in *S. fusiforme* shoots are presented in this study, because the responses to abiotic factors were similar among these pigment contents but were different among the ratio of these pigments to Chl *a* content in the kelp *U. pinnatifida* [29].

2.4. Statistical Analysis

Differences in the initial wet weight of *S. fusiforme* shoots among eight different treatments were analyzed by analysis of variance (ANOVA). The combined effects of temperature, nutrients, and irradiance on the SGR and biochemical compositions were evaluated by three-way ANOVA. When significant interactions between two or three factors were found, Tukey's multiple comparison tests were utilized to ascertain if the non-additive effect was synergistic or antagonistic. Normality and homoscedasticity of the data were checked using the Shapiro–Wilk test and bartlett test, respectively. Some data (Fuco/Chl *a* and Nitrogen) were transformed to the logarithm in order to stabilize variance. All analyses were performed using John's Macintosh Project (JMP) software version 10 (SAS, Cary, NC, USA).

3. Results

The mean initial wet weight (±standard deviation (SD)) of shoots was 1.346 ± 0.205 g. There were no significant differences in initial wet weight among treatments (df = 7, MS = 0.103, $F = 3.304$, $p > 0.05$). Mean (±SD) initial carbon and nitrogen contents were 22.827 ± 1.293% and 1.233 ± 0.135%, respectively. Mean (± SD) initial Chl *a*, Chl c_2/Chl *a*, Fuco/Chl *a*, and VAZ/Chl *a* were 0.349 ± 0.009, 0.122 ± 0.014, 0.236 ± 0.022, and 0.225 ± 0.031 mg g^{-1}wwt, respectively. Nitrate concentration in 5% PESI was 38.060 ± 0.435 µM, whereas that in AW was less than 1.610 µM.

ANOVA detected significant individual effects of temperature and irradiance, and their significant interaction on SGR (Table 1). Tukey's multiple comparison test indicated that the SGR significantly increased in response to elevated irradiance at 23 °C, but did not change at 26 °C (Figure 1A). SGR decreased in response to elevated temperature in high irradiance treatments but not in low irradiance treatments.

Table 1. Results of three-way ANOVA on the effects of temperature, nutrient availability, and irradiance on the specific growth rate of *Sargassum fusiforme*. Sources that are significant at $p < 0.05$ and $p < 0.01$ are denoted as '*' and '**' respectively.

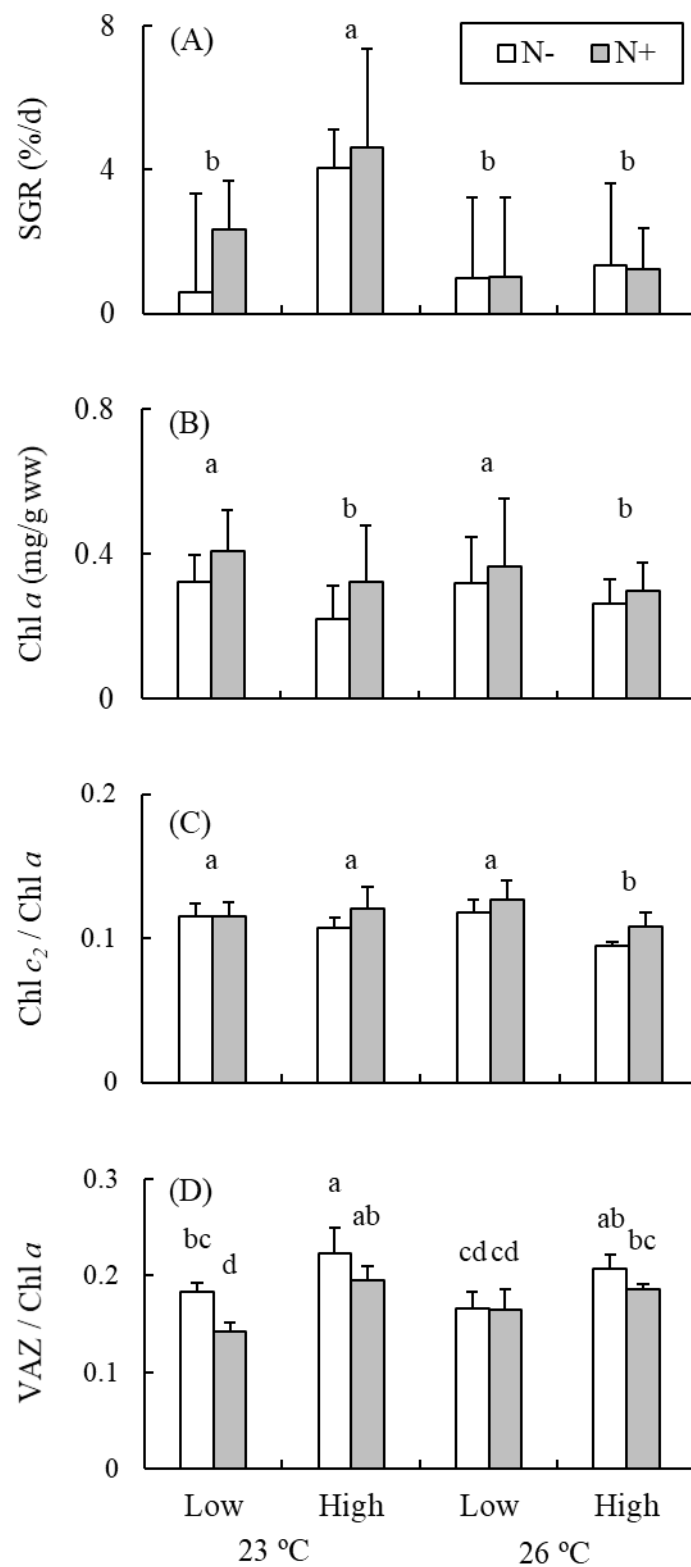| Source	df	MS	F	p	
Temperature (T)	1	36.920	8.628	0.005	**
Nutrient (N)	1	3.710	0.866	0.358	
Irradiance (I)	1	29.960	7.001	0.012	*
T × I	1	20.240	4.730	0.036	*
T × N	1	4.110	0.961	0.333	
I × N	1	1.200	0.281	0.599	
T × N × I	1	0.800	0.187	0.668	

Figure 1. Specific growth rate (**A**), Chl *a* content (**B**), Chl c_2 / Chl *a* ratio (**C**), and VAZ/Chl *a* ratio (**D**) of *Sargassum fusiforme* shoots cultured in eight different treatments (mean + SD, $n = 6$). Low and High indicate low (30 μmol photon m^{-2} s^{-1}) and high (150 μmol photon m^{-2} s^{-1}) irradiance treatments, respectively. N+ and N- indicate nutrient enriched and non-enriched treatments, respectively. Different small letters such as a, b, ab, bc, cd, d, bc denote statistical significances among different treatments where $p < 0.05$.

For pigments, Chl *a* content was significantly affected by irradiance (Table 2), and was higher at low than at high irradiance levels (Figure 1B). Chl c_2/Chl *a* was affected by irradiance and nutrients with an interaction between irradiance and temperature (Table 2). Tukey's test found that Chl c_2/Chl *a* decreased in response to elevated irradiance at 26 °C but not at 23 °C (Figure 1C). Chl c_2/Chl *a* also decreased in response to elevated temperature in high irradiance treatments, but not in low irradiance treatments. Additionally, Chl c_2/Chl *a* increased by nutrient enrichment. ANOVA did not detect any significance effects on Fuco/ Chl *a* (Table 2). VAZ/Chl *a* was significantly affected by irradiance, nutrients, and interaction between temperature and nutrients (Table 2). There was also an interaction between these three factors. VAZ/Chl *a* increased in response to elevated irradiance (Figure 1D). VAZ/Chl *a* decreased by nutrient enrichment in the low temperature combined with low irradiance treatment, whereas this effect was not detected in other treatments.

Table 2. Results of three-way ANOVA on the effects of temperature, nutrients, and irradiance on photosynthetic pigment content of *Sargassum fusiforme*. Sources that are significant at $p < 0.05$, $p < 0.005$ and $p < 0.001$ are denoted as '*', '**' and '***' respectively.

Source	df	MS	F	p	
Chl *a*					
Temperature (T)	1	0.001	0.005	0.829	
Nutrient (N)	1	0.055	3.879	0.056	
Irradiance (I)	1	0.074	5.239	0.027	*
T × I	1	0.003	0.208	0.651	
T × N	1	0.008	0.540	0.467	
I × N	1	<0.001	0.001	0.973	
T × N × I	1	0.001	0.055	0.816	
Chl c_2 /Chl *a*					
Temperature (T)	1	<0.001	0.952	0.335	
Nutrient (N)	1	0.001	9.593	0.004	**
Irradiance (I)	1	0.002	15.402	<0.001	***
T × I	1	0.001	11.380	0.002	**
T × N	1	0.001	0.677	0.415	
I× N	1	<0.001	2.405	0.129	
T × N × I	1	<0.001	0.817	0.371	
Fuco/Chl *a*					
Temperature (T)	1	<0.001	0.094	0.761	
Nutrient (N)	1	<0.001	0.151	0.700	
Irradiance (I)	1	<0.001	0.142	0.708	
T × I	1	0.001	0.671	0.418	
T× N	1	<0.001	0.134	0.716	
I× N	1	0.001	0.675	0.416	
T × N× I	1	<0.001	<0.001	0.985	
VAZ/Chl *a*					
Temperature (T)	1	0.003	0.483	0.491	
Nutrient (N)	1	0.187	26.901	<0.001	***
Irradiance (I)	1	0.547	78.877	<0.001	***

Table 2. *Cont.*

Source	df	MS	F	p	
T × I	1	0.019	2.673	0.110	
T × N	1	0.054	7.734	0.008	**
I × N	1	<0.001	0.024	0.877	
T × N × I	1	0.036	5.165	0.029	*

Carbon content was significantly affected by temperature, irradiance, and by the interaction between temperature and nutrients. Nitrogen content was significantly affected by nutrients and interaction between nutrients and irradiance (Table 3). Tukey's test showed that carbon content decreased in response to elevated temperature in non-enriched treatments but not in nutrient-enriched treatments (Figure 2A). Nitrogen content increased in response to nutrient enrichment at high irradiance but not at low irradiance (Figure 2B).
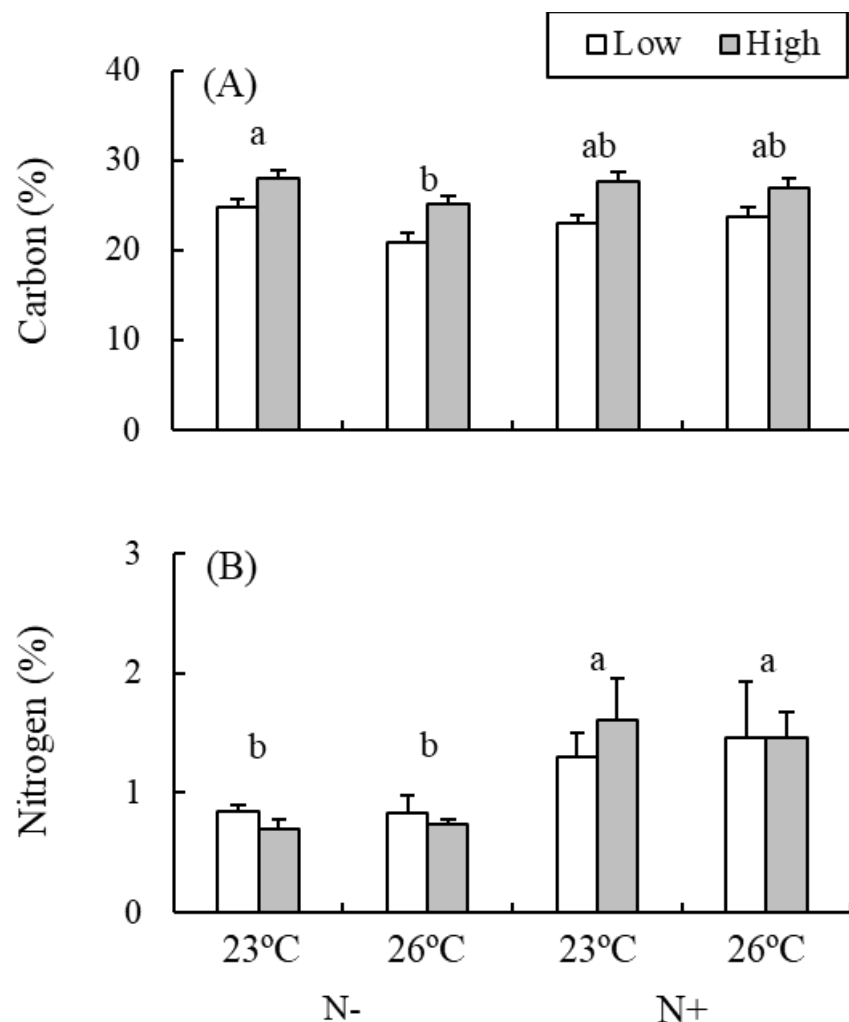

Figure 2. Carbon (**A**) and nitrogen (**B**) contents of *Sargassum fusiforme* shoots cultured in eight different treatments (mean + SD, *n* = 6). Low and High indicate low (30 μmol photon m^{-2} s^{-1}) and high (150 μmol photon m^{-2} s^{-1}) irradiance treatments, respectively. N+ and N- indicate nutrient enriched and non-enriched treatments, respectively. Different small letters indicate statistical significances among different treatments *p* < 0.05.

Table 3. Results of three-way ANOVA on the effects of temperature, nutrient availability, and irradiance on biochemical composition of *Sargassum fusiforme*. Sources that are significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$ are denoted as '*', '**' and '***' respectively.

Source	df	MS	F	p	
Carbon %					
Temperature (T)	1	40.829	9.413	0.004	**
Nutrient (N)	1	2.517	0.580	0.451	
Irradiance (I)	1	192.871	44.469	<0.001	***
T × I	1	0.041	0.009	0.923	
T × N	1	25.426	5.863	0.020	*
I × N	1	1.350	0.311	0.580	
T × N × I	1	2.403	0.554	0.461	
Nitrogen %					
Temperature (T)	1	<0.001	0.032	0.859	
Nutrient (N)	1	0.859	174.838	<0.001	***
Irradiance (I)	1	0.001	0.184	0.670	
T × I	1	0.001	0.233	0.632	
T × N	1	<0.001	0.002	0.964	
I × N	1	0.038	7.772	0.008	**
T × N × I	1	0.010	1.961	0.169	

4. Discussion

Significant interactive effects of temperature and nutrient availability on macroalgal growth, which indicate the negative effect of warming is synergized or antagonized by nutrient enrichment, have previously been detected in the kelps *Eisenia bicyclis* [18] and *Saccharina japonica* [19], and in sexually produced propagules (i.e., early life stages) of the fucoid *S. fusiforme* [24]. In contrast, several studies have shown significant individual effects of temperature and nutrients on growth without their interaction, which indicates the additive effect of these factors in the kelps *Ecklonia cava* [36], *Laminaria ochroleuca* [37], and *Macrocystis pyrifera* [26], and holdfasts of *S. fusiforme* [24]. However, in the present study, a significant interaction between warming and nutrient enrichment, as well as a significant individual effect of nutrient enrichment on growth, were not detected in *S. fusiforme* shoots. This coincides with the results obtained from shoots of other fucoid species, namely, *S. patens* [51] and *Phyllospora comosa* [33]. Thus, responses to the combined effects of temperature and nutrients are different among life stages and body parts in *S. fusiforme*, and appear to differ between kelp and fucoid species; although, this might also depend on species and experimental conditions.

Instead of the temperature and nutrient interaction, the present study detected a significant interaction between temperature and irradiance affecting the growth of *S. fusiforme*. This indicated that the elevated temperature decreased SGR in the higher irradiance treatments but not in the lower ones, and that the elevated irradiance increased SGR at 23 °C but not at 26 °C. These results suggest that the negative effect of warming on *S. fusiforme* growth was synergized by elevated irradiance, and the positive effect of the elevated irradiance was antagonized by the warming. Similarly, significant interactive effects of temperature and irradiance were found on Chl *c*/Chl *a*, which decreased in response to warming at the higher irradiance level but not at the lower one. These results can be explained by the fact that excess light energy under environmental stress (i.e., warm temperature, in this case) resulted in an increase in the production of reactive oxygen species in chloroplasts, which caused photoinhibition and chlorophyll degradation [11,12,25]; although, the

present study did not show maximum efficiency of photosystem II (*Fv/Fm*) as evidence of photoinhibition. On the other hand, Chl *a* content of *S. fusiforme* decreased in response to elevated irradiance, but this negative effect was not synergized by elevated temperature in the present study. Hence, warming combined with strong irradiance might decrease *S. fusiforme* growth via Chl *c* degradation rather than Chl *a* degradation; although, this hypothesis needs further verification.

Endo et al. [29] showed that elevated irradiance combined with decreased temperature (from 15 to 5 °C) caused a decrease in growth rate and an increase in ratio of xanthophyll cycle pigments to Chl *a* in the kelp *U. pinnatifida*. This implies that excess light energy combined with environmental stress (i.e., cold temperature, in this case) might result in photoinhibition and therefore lead to photoprotective response in this alga. In the present study, interactive effects of elevated irradiance and warming were evident in *S. fusiforme* growth, but not in VAZ/Chl *a* (i.e., ratio of xanthophyll cycle pigments to Chl *a*). Instead, a complex interaction was detected among temperature, nutrients, and irradiance, as well as individual effects of irradiance and nutrients on VAZ /Chl *a*. This interaction indicated that VAZ/Chl *a* increased in response to elevated irradiance in most treatments but not in those combining higher temperature with nutrient enrichment. This implies that high light acclimation in VAZ/Chl *a* might be suppressed by warming and eutrophication. Additionally, VAZ/Chl *a* decreased in response to nutrient enrichment in the lower temperature combined with lower irradiance treatment, whereas it was not affected by nutrient enrichment in the higher temperature and irradiance treatment. Hence, the negative effect of nutrient enrichment on VAZ/Chl *a* might be antagonized by warming and elevated irradiance; although, further evaluation is needed to confirm reproducibility of these results.

Endo et al. [18] showed that the positive effect of elevated irradiance on carbon content was synergized by reduced nutrient availability in the kelp *E. bicyclis*. This result can be supported by a previous study showing increases in some tricarboxylic acid cycle intermediates, such as succinate, malate, and fumarate, in response to nitrogen starvation in a cyanobacterial species using a metabolomics approach [53]. In contrast, the present study found a significant individual effect of temperature on the carbon content of *S. fusiforme*, as well as a significant interaction between temperature and nutrient enrichment. Nevertheless, previous studies have rarely detected the effects of temperature and nutrients on the carbon contents of kelp and fucoid species [18,26,32–34]. In the present study, carbon content decreased in response to warming in the non-enriched treatment, while it was not affected by temperature in the enriched treatment. This indicated that the negative effect of warming on carbon content was antagonized by nutrient enrichment. The physiological mechanism behind this result is unknown but is expected to be clarified by metabolomics.

In the present study, nitrogen content of *S. fusiforme* increased in response to nutrient enrichment but was not affected by temperature elevation. This coincides with most of previous results reported on kelp [18,19,26,36,37] and fucoid species [34,35]; although, some studies showed that elevated temperature caused a decrease and an increase in nitrogen contents in the fucoid *Sargassum flavicans* [32] and the kelp *Eisenia bicyclis* [25], respectively. Additionally, in the present study, elevated irradiance had no effect on the nitrogen content of *S. fusiforme*, whereas it decreased the concentration of this element in the kelp *E. bicyclis* [18]. Therefore, the susceptibility of nitrogen content to irradiance may differ between kelp and fucoid species.

The present study revealed that the negative effect of warming on the growth of *S. fusiforme* was antagonized by decreased irradiance. Based on this result, it is predicted that heatwaves might suppress the growth of this species growing at shallower depths, but it will have little effect at deeper depths. Although the latter is reported to represent a refuge from warming for a coral community [54], *S. fusiforme* commonly grows only between the lower intertidal and shallow subtidal zones [47], and therefore the heatwaves may be a serious threat for this species. The present study also showed that the negative effect of warming on the carbon content of *S. fusiforme* was antagonized by nutrient en-

richment. Therefore, carbon fixation and sequestration by this species may be affected by local nutrient environments under ocean warming. Moreover, this finding indicates that nutrient enrichment may contribute to carbon fixation and sequestration, not only by enhancing the recruitment and growth of macroalgae [19,24,36], but also by maintaining a high macroalgal carbon content. In situ nutrient enrichment in coastal waters may also decrease light availability for benthic macroalgae, which antagonizes the negative effect of warming through phytoplankton blooms. However, it is difficult to determine the adequate level of nutrient enrichment in order to enhance macroalgal production without causing any negative effect on the surrounding coastal ecosystems. The introduction of fish or shellfish aquaculture facilities near macroalgal forests is another measure that can be used to increase nutrient loading [16]; although, this does not seem feasible in wave-exposed coasts, where macroalgal forests are often found. Nevertheless, the results of the present study suggest that the management of local light and nutrient environments is significant in order to antagonize the negative effect of heatwaves on *S. fusiforme*, which is an ecologically and economically important species. Further studies on the optimal irradiance and nutrient concentration for growth and biochemical compositions of this species under ocean warming may provide insight for the restoration and conservation of marine macroalgal forests dominated by *S. fusiforme.*

Author Contributions: Conceptualization, H.E., Y.S. and M.N.A.; methodology, H.E. and Y.S.; software, H.E. and H.C.; validation, H.E., Y.S. and H.C.; formal analysis, H.E. and H.C.; investigation, H.C., E.I. and Y.O.; resources, M.N.A.; data curation, H.E. and H.C.; writing—original draft preparation, H.E. and H.C.; writing—review and editing, H.E., Y.S. and H.C.; visualization, H.E. and Y.S.; supervision, H.E., Y.S. and M.N.A.; project administration, H.E. and M.N.A.; funding acquisition, M.N.A. and H.E. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data and any statistical analysis can be made available from the corresponding author upon request.

Conflicts of Interest: Author Yoichi Sato was employed by the company Riken Food Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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