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Ocean acidification increases iodine accumulation in kelp-based coastal food webs

Running head: Ocean acidification increases iodine in kelp

Dong Xu1,2,*, Georgina Brennan3,*, Le Xu1, Xiao W. Zhang1, Xiao Fan1, Wen T. Han1, Thomas Mock4, Andrew McMinn5,6, David A. Hutchins7, Naihao Ye1,2,*

1Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao, China

2Function Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Qingdao, China

3Molecular Ecology and Fisheries Genetics Laboratory, School of Biological Sciences, Bangor University, Bangor LL57 2UW, UK

4School of Environmental Sciences, University of East Anglia, Norwich Research Park, Norwich, United Kingdom

5Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Tasmania, Australia

6Fisheries College, Ocean University of China, Qingdao, China

7Department of Biological Sciences, University of Southern California, Los Angeles, CA, USA

*These authors contributed equally to this work.

Correspondence: Naihao Ye, tel. 86-532-85830360, fax 86-532-85830360, e-mail: yenh@ysfri.ac.cn.

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ABSTRACT

Kelp are main iodine accumulators in the ocean, and their growth and photosynthesis are likely to benefit from elevated seawater CO$_2$ levels due to ocean acidification. However, there are currently no data on the effects of ocean acidification on iodine metabolism in kelp. As key primary producers in coastal ecosystems worldwide, any change in their iodine metabolism caused by climate change will potentially have important consequences for global geochemical cycles of iodine, including iodine levels of coastal food webs that underpin the nutrition of billions of humans around the world. Here, we found that elevated $p$CO$_2$ enhanced growth and increased iodine accumulation not only in the model kelp *Saccharina japonica* using both short-term laboratory experiment and long-term *in situ* mesocosms, but also in several other edible and ecologically significant seaweeds using long-term *in situ* mesocosms. Transcriptomic and proteomic analysis of *Saccharina japonica* revealed that most vanadium-dependent haloperoxidase genes involved in iodine efflux during oxidative stress are down-regulated under increasing $p$CO$_2$, suggesting that ocean acidification alleviates oxidative stress in kelp, which might contribute to their enhanced growth. When consumed by abalone (*Haliotis discus*), elevated iodine concentrations in *S. japonica* caused increased iodine accumulation in abalone, accompanied by reduced synthesis of thyroid hormones. Thus, our results suggest that kelp will benefit from ocean acidification by a reduction in environmental stress however, iodine levels in kelp-based coastal food webs will increase, with potential impacts on biogeochemical
cycles of iodine in coastal ecosystems.

INTRODUCTION

Anthropogenic emissions of CO₂, associated ocean acidification (OA) and global warming, are increasing at rates unprecedented in the geological record (Sunday et al., 2017; Thomsen et al., 2017). These changes are expected to directly affect primary producers by changing growth rates, photosynthesis and metabolism, and indirectly by reducing biodiversity and altering ecosystem structure (Enochs et al., 2015; Martínez-Botí et al., 2015; Myers et al., 2017; Ullah, Nagelkerken, Goldenberg, & Fordham, 2018). Furthermore, OA is predicted to cause an increase in the accumulation of toxic phenolic compounds across multiple trophic levels, from phytoplankton to zooplankton (Jin et al., 2015). Higher temperatures are also expected to increase the concentrations of nutrients such as nitrogen (N), potassium (K), and magnesium (Mg) stored in living biomass (Zhang et al., 2018) and impact the global biotic metabolic rates (Dillon, Wang, & Huey, 2010). Metabolic rate changes and nutrient variation under climate change will affect human health through consumption of these organisms (Jin et al., 2015; McKibben et al., 2017; Zhu et al., 2018) and potentially impact food security (Bloom, Burger, Asensio, & Cousins, 2010; Loladze, 2002; Myers et al., 2017; Phalkey, Aranda-Jan, Marx, Höfle, & Sauerborn, 2015). However, climate impacts on the nutrient composition of primary producers (such as, seaweeds) in coastal ecosystem has, to date, been little explored.
Seaweeds, including, brown, red, and green algae are a rich source of iodine (Küpper, 2015; Küpper et al., 2008; Nitschke & Stengel, 2015; Ye et al., 2015) and are widely harvested for food and exploited for commercial alginate and iodine. Iodine is an essential nutrient required for the synthesis of thyroid hormones (THs), triiodothyronine (T3) and thyroxine (T4) (Berg et al., 2017). Iodine deficiency in humans can result in unexpected health problems such as hypothyroidism and goiter, whereas high iodine intake from seaweeds can lead to hyperthyroidism, a reversible condition which can cause symptoms such as nontoxic or diffuse nodular goiter, latent Graves’ disease and long standing iodine deficiency (Leung & Braverman, 2014).

Natural consumers of seaweeds such as fish and shellfish are also a rich dietary source of iodine for humans (Nitschke & Stengel, 2015). It is therefore essential to understand how the iodine content of seafood will change under global climate change. This information can for instance be used by the World Health Organization (WHO) to provide recommendations on appropriate levels of seaweeds consumption to maintain a sufficient daily iodine intake (Fig. 1).

With respect to iodine, kelp (order Laminariales) are particularly important as they are not only being the greatest iodine accumulators among living organisms, but also playing an important role in the global biogeochemical cycle of iodine (Küpper, 2015; Küpper et al., 2008). In *Laminaria* tissue, iodine is mostly stored as iodide on the thallus surface and in the apoplast. Iodide efflux occurs under oxidative stress and iodide in the peripheral tissues acts as an inorganic antioxidant to detoxify both aqueous oxidants.
and ozone, stimulating the release of molecular iodine and volatile iodinated compounds to the atmosphere (Cosse et al., 2009; Küpper & Kroneck, 2014). Oxidative burst and associated iodine metabolism also play a direct defensive role in both controlling the growth of potentially pathogenic bacteria living at the thallus surface and scavenging a variety of reactive oxygen species (ROS) (Küpper et al., 2008; Küpper, Müller, Peters, Kloareg, & Potin, 2002; Strittmatter et al., 2016). During oxidative burst, algal cells rapidly release large amounts of activated oxygen species (AOS), such as superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) or hydroxyl radicals (OH$^-$). This release is elicited by exposure to oligomeric degradation products of alginate and possibly other molecular signals. It has been suggested that the mechanism of iodine antioxidation is linked to the production of vanadium-dependent haloperoxidases (vHPOs), which comprises seventeen vanadium-dependent bromoperoxidases (vBPOs) and fifty-nine iodoperoxidases (vIPOs) in the S. japonica genome (Butler & Carter-Franklin, 2004; Cosse et al., 2009; Ye et al., 2015) (Fig. 1).

Despite widespread interest in the biological response of kelp to climate change, there is currently no information on the in situ molecular response associated with iodine metabolism. Here, three ecologically and socio-economically important kelp species (S. japonica, U. pinnatifida, and M. pyrifera), as well as another four coastal seaweeds (Ulva pertusa and Ulva intestinalis in Chlorophyta; Gracilaria lemaneiformis and Gracilaria chouae in Rhodophyta) were used to study the effect of increasing pCO$_2$ on iodine accumulation of seaweeds using both short-term laboratory experiments and one
long-term, in situ experiment. In a feeding experiment, the transfer of iodine between 
*S. japonica* and its consumer *Haliotis discus* was investigated. We used these 
experiments to explore: (i) the effect of elevated \( p\text{CO}_2 \) on iodine accumulation in kelp-
based coastal food webs; (ii) whether short-term laboratory experiments can be 
compared with in situ ocean mesocosms; (iii) the molecular mechanism involved in 
iiodine metabolism at the transcriptional and protein level in response to elevated \( p\text{CO}_2 \). 
Together, the research presented here will be used to assess the global biogeochemical 
cycle of iodine under future climate change and provide information for making 
recommendations on appropriate levels of seaweeds consumption to reach adequate 
daily iodine intake.

**MATERIALS AND METHODS**

**Algal material and culture conditions**

For the laboratory experiments, young sporophytes of *S. japonica* were collected from 
semi-enclosed Sungo Bay, located on the northwestern coast of the Yellow Sea, China 
(37°01′–37°09′ N, 122°24′–122°35′ E) in December 2016, when the mean seawater 
temperature was 10.3°C. Similar sized algal samples (average length is 10 cm) were 
transported back to the laboratory within three hours, in a tank of cold seawater. In the 
laboratory, the intact samples were washed with sterile seawater until they were free 
from visible epiphytes and then pre-cultured in aquaria supplemented with f/2 medium 
(Guillard, 1975) at 10 ± 1°C with vigorous air bubbling for 2 days before the start of 
the experiment. The lighting conditions were set at 100 µmol photons m\(^{-2}\) s\(^{-1}\) supplied
by white fluorescent lamps, with a photoperiod of 12 h light and 12 h darkness.

Effect of increasing $pCO_2$ and temperature on iodine accumulation

To study the individual effect of temperature on iodine accumulation in *S. japonica* sporophytes, a gradient of five temperatures (5°C, 10°C, 15°C, 20°C, and 23°C) was established, mimicking the annual variation of temperature during the growth period. To examine the combined effect of increasing $pCO_2$ and temperature, three temperatures (10°C, 15°C, 20°C) and five $pCO_2$ levels (400 μatm, 700 μatm, 1,000 μatm, 1,500 μatm, and 2,000 μatm), were selected (Table S1). These $pCO_2$ levels were chosen as they reflect current and future $pCO_2$ levels up to the year 2,300 under IPCC (Intergovernmental Panel on Climate Change) scenario RCP 8.5. The experiment was conducted in flasks using three biological replicates per treatment. Before experiments, algal samples were pre-cultured in seawater under various $pCO_2$ and temperature conditions for 96 hours. Pre-cultured algae were then inoculated into 500-mL Erlenmeyer flasks containing 400 mL of adjusted f/2 seawater medium and supplemented with 20 μmol L$^{-1}$ KI (half saturation concentration derived from iodine uptake kinetics, in Fig. S1) for 24 h. At the end of the experiment, algal samples in each treatment were collected and rinsed for iodine determination within 24 h. Individual flasks were cultured inside a CO$_2$ chamber (HP1000G-D, China), programmed to supply 400 μatm, 700 μatm, 1,000 μatm, 1,500 μatm, or 2,000 μatm $pCO_2$ by bubbling at each designated temperature for 24 h. The pH and temperature were measured at the beginning and end of the experiment with a pH meter (Orion ROSS, Fisher Scientific.
Instruments). To determine total alkalinity (TA) at each treatment, 20 ml of culture medium was filtered with GF/F membrane and measured using an 848 Titrino plus automatic titrator (Metrohm, Riverview, FL, USA). Chemical carbonate system parameters were calculated using the CO2SYS Package in MS Excel (Pierrot, Lewis, & Wallace, 2006) based on pH, temperature, salinity, and TA (Table S1).

Potential antioxidant property of iodide in *S. japonica*

To determine the potential antioxidant properties of iodide in *S. japonica*, oligoguluronate elicitor (GG, Shanghai Zzbio Co, Ltd, China) was used as exogenous defense elicitors to induce oxidative stress and iodine efflux from algal tissue into the culture medium (referred to previous study of (Küpper, Kloareg, Guern, & Potin, 2001). Algal samples were inoculated into 150-mL Erlenmeyer flasks containing 100 mL sterile seawater without (set as control) or with exogenous oligoguluronate elicitors at a final concentration of 100 μg ml⁻¹ (GG, set as treatment). Three elicitation experiments were conducted at 10°C and 100 μmol photons m⁻² s⁻¹. To understand the effect of GG on iodine efflux over time, three seawater samples were randomly taken from the untreated control and GG treatments at four time intervals (0 h, 1 h, 3 h, and 5 h). To understand the effect of elevated *p*CO₂ in the presence of GG, iodine efflux was measured after 3 hours under five *p*CO₂ conditions (400 μatm, 700 μatm, 1,000 μatm, 1,500 μatm, or 2,000 μatm, bubbled at 10°C for 24 h). To investigate the prolonged effect of ocean acidification on iodine efflux, the short-term vs long-term response of iodine efflux in *S. japonica* under ocean acidification conditions was compared. Under
short-term-ambient-carbon (SAC) and short-term-elevated-carbon (SEC) treatments, algae were pre-cultured at CO$_2$ concentrations of 400 $\mu$atm and 1,000 $\mu$atm respectively, for 7 days. Under long-term-ambient-carbon (LAC) or long-term-elevated-carbon (LEC) treatments, algae were pre-cultured under the same $p$CO$_2$ levels for 30 days. Following pre-culturing, iodine efflux under ambient (400 $\mu$atm) or elevated (1,000 $\mu$atm) $p$CO$_2$ was measured after 3 hours.

**In situ mesocosm experiments**

Three kelp species, *S. japonica*, *U. pinnatifida*, and *M. pyriforma*, as well as other coastal seaweeds (*Ulva pertusa* and *Ulva intestinalis* in Chlorophyta; *Gracilaria lemaneiformis* and *Gracilaria chouae* in Rhodophyta) were collected off the coastline of Sungo Bay for the mesocosm experiment (Fig. S2). With the exception of *M. pyriforma*, these algae are widely consumed by humans. *M. pyriforma* was selected as it is a preferred source of marine invertebrates, such as sea urchins and abalone, which are harvested by the local fishing industry. The mesocosms were designed following (Xu et al., 2017). Six net cages (three per treatment) were used, applying two $p$CO$_2$ levels, ambient $p$CO$_2$ (400 $\mu$atm, bubbled with air) and elevated $p$CO$_2$ (1,000 $\mu$atm, bubbled with air/CO$_2$ premixed gas using a CO$_2$ Enrichlor, CE-100B; Wuhan Ruihua Instrument & 25 Equipment Ltd) (Fig. S3). One hundred and twenty individuals of each algal species of similar size were grown in each cage for approximately five months, from December 2016 to May 2017. During this period, six individuals of each algal species from each net cage (n=6) were randomly collected for iodine determination, and 10 individuals
(n=10) from each net cage were selected and weighed to monitor growth. To determine
the effect of kelp size on iodine accumulation during the culture period, kelp which
were pre-acclimated under ambient or elevated $pCO_2$ for 48 hours in the sea were
sampled at different time points: 0 day, 30 days, 60 days, and 90 days for $S. japonica$,
0 day, 30 days, and 60 days for $U. pinnatifida$, and 0 day, 20 days, and 40 days for $M.$
pyrifera. Seawater carbonate chemistry in the ambient (400 μatm) or elevated (1,000
μatm) $pCO_2$ culture environments were monitored every two days and the carbonate
chemistry was calculated using the CO2SYS Package in MS Excel based on pH,
temperature, salinity, and TA (Fig. S4) (for further details of the mesocosm design, see
methods in supporting information).

To assess the effects of consumption of $S. japonica$ by abalone, sporophytes were grown
at either ambient (400 μatm) or elevated (1,000 μatm) $pCO_2$. Five feeding experiments
were conducted in situ: 1) abalones were fed with ambient $pCO_2$-cultured $S. japonica$
and were also cultured in an ambient $pCO_2$-cage; 2) abalones were fed with ambient
$pCO_2$-cultured $S. japonica$ and were cultured in an elevated $pCO_2$-cage; 3) abalones
were fed with elevated $pCO_2$-cultured $S. japonica$ and were also cultured in an elevated
$pCO_2$-cage; 4) abalones were fed with elevated $pCO_2$-cultured $S. japonica$ and were
cultured in an ambient $pCO_2$-cage; 5) abalones were fed with ambient $pCO_2$-cultured $S.$
$japonica$ and were cultured in the sea with no cage; 6) abalones were fed with elevated
$pCO_2$-cultured $S. japonica$ and were cultured in the sea with no cage. The experiments,
with six replicates (n = 6), were conducted independently for 30 days, starting on April
10th 2017. The experimental abalones were fed with fresh algal tissue at intervals of 3 days and collected for iodine determination at intervals of 10 days. At the end of the experiments, the abalones were cleaned with sterile seawater and the tissue was removed quickly and entirely from the shell, weighed and frozen in liquid nitrogen, and stored at -80 °C for total iodine and thyroid hormones (THs) determination (see methods in supporting information).

Transcriptomic and proteomic analysis of iodine metabolism in S. japonica

To explore the effect of ocean acidification on the iodine metabolism on the S. japonica transcriptome, on day 90 of the mesocosm experiment, S. japonica sporophytes were randomly selected, cleaned with sterile seawater, frozen in liquid nitrogen and stored at -80 °C for subsequent transcriptomic (with three biological replicates for each $pCO_2$) and proteomic (with two biological replicates for each $pCO_2$) analysis (see methods in supporting information).

Statistical analysis

Statistical models were carried out with R software (R Core Development Team, 2014) and model selection was based by Akaike Information Criterion (AIC). The effect of temperature and the combined effect of increasing $pCO_2$ and temperature on iodine accumulation were analyzed using a mixed effects model using the lmer function within the package lme4 and lmerTest. When modelling the effect of temperature alone, temperature was used as a fixed factor, when modelling the combined effect of
temperature and $pCO_2$, both temperature and $pCO_2$ were used as fixed factors (the interaction between temperature and $pCO_2$ was dropped from the model based on AIC).

The effect of measurement type, either iodide or total iodine accumulation, were used as random factors in both models.

Using the `lm` function within R, three linear models were used to compare changes in iodine efflux in *S. japonica* treated with oligoguluronates (GG) or untreated (control).

To test for the effect of time (hours) on iodine efflux, time, treatment (GG or control), and the interaction between time and treatment were included in a linear model. The effect of increasing $pCO_2$ (400 to 2000 µatm), treatment (GG or control), and the interaction between $pCO_2$ and treatment were included in a second linear model. The effect of ambient and elevated $pCO_2$, treatment (GG or control), time (days), the interaction between $pCO_2$ and time, and the interaction between treatment and $pCO_2$ were included in a third linear model.

In order to compare the responses of the three species of seaweeds used in the mesocosm experiment (*S. japonica, M. pyrifera* and *U. pinnatifida*), the relative change in iodine accumulation and weight (g) over time were calculated, relative to the responses at time point zero, under control conditions (400 µatm). To test for the effect of $pCO_2$ (ambient or elevated), over time (days), on relative changes in iodine accumulation, the effect of $pCO_2$, time, species and the interactions between time and species were included in a linear model. To test for the effect of $pCO_2$, over time, on
the relative weight of the seaweeds, the effect of $p$CO$_2$, time, species and the interaction between $p$CO$_2$, time and species were included in a linear model.

Three linear models were used to test the effect of diet and $p$CO$_2$ on 1) iodine enrichment 2) T3 concentration and 3) T4 concentration in the abalone (*H. discus*). For iodine enrichment, the effect of diet, time, $p$CO$_2$, the interaction between diet, time and $p$CO$_2$, and environment (caged or non-caged) were included in the model. For both T3 and T4 hormones, the effect diet, $p$CO$_2$, environment (caged or non-caged) and the interaction between environment and diet were included in the model. Data collected for iodine enrichment and T3 concentration best fit a lognormal distribution and for this reason a generalized linear model which takes into consideration a lognormal distribution was carried out, using the glm function in the stats package.

**RESULTS**

**Effect of increasing $p$CO$_2$ and temperature on iodine accumulation of *S. japonica* in laboratory**

Elevated $p$CO$_2$ caused changes in iodine accumulation in *S. japonica* when both the $p$CO$_2$ and temperature were altered at the same time (Fig. 2a; $F_{1,86} = 14.515$, $P = 0.0003$), with no significant effect of temperature on iodine accumulation (Fig. 2a; $F_{1,86} = 1.507$, $P = 0.223$). As $p$CO$_2$ increased from 400 µatm to 1,000 µatm at the control temperature (10 ºC), both iodide and total iodine increased and reached the highest observed iodine accumulation at 1,000 µatm. As $p$CO$_2$ increased to 1,500 µatm, iodide and total iodine
accumulation decreased and leveled off as $pCO_2$ increased to 2,000 µatm. When temperature was altered in isolation, there was a significant effect on iodide and total iodine accumulation in *S. japonica* (Fig. 2b; $F_{1,27} = 19.47$, $P = 0.0002$). As temperature increased, from 5 ºC to 15 ºC under the control $pCO_2$ concentration (400 µatm), iodide and total iodine accumulation increased by 64% and 58%, respectively (Fig. 2b). At 20 ºC and 23 ºC, iodine accumulation decreased slightly but it was not significantly different from the maximum iodine accumulation observed at 15 ºC. However, there were no significant effects of increasing $pCO_2$ or temperature (isolation or combination) on iodine solubility and availability in seawater medium (Fig. S5).

**Potential antioxidant properties of iodide in *S. japonica* in laboratory**

Under oxidative stress, induced by the addition of oligoguluronates (GG), iodine efflux in *S. japonica* was 61% greater than the untreated control group, after 5 hours (Fig. 3a; $F_{1,225} = 22.428$, $P = 0.0001$). Iodine efflux in *S. japonica* treated with GG increased over three hours and then decreased slightly after five hours, with little change in the untreated control group over time (Fig. 3a; *effect of time on the iodine efflux*, $F_{1,47} = 4.685$, $P = 0.0427$) and is supported by a significant interaction between the treatment (GG and control) and time (Fig. 3a; $F_{1,20} = 4.562$, $P = 0.0452$).

Iodine efflux in *S. japonica* increased with increasing $pCO_2$ until 1500 µatm and decreased again at 2000 µatm (Fig. 3b; $F_{1,10} = 2.95$, $P = 0.098$). Iodine efflux was 51% - 72% higher in *S. japonica* treated with GG than the untreated group, depending on the
\( pCO_2 \) levels (Fig. 3b; \( F_{1,475} = 148.375, P < 0.0001 \), and this is supported by a significant interaction between the \( pCO_2 \) conditions and treatment (GG and untreated) (Fig. 3b; \( F_{1,17} = 5.173, P = 0.031 \)).

Long-term exposure to elevated \( pCO_2 \) (LEC) intensified iodine efflux from \textit{S. japonica}. Iodine efflux from \textit{S. japonica} treated with GG was 29% higher after 30 days exposure to elevated \( pCO_2 \), compared with 7 days of exposure to elevated \( pCO_2 \) (SEC) and 44% greater than both short- and long-term exposure to ambient \( pCO_2 \) conditions (7 days and 30 days; SAC and LAC) (Fig. 3c; \( F_{1,48} = 21.119, P = 0.0002 \)). Very little change in iodine efflux was observed in the untreated control group (Fig. 3c; \( F_{1,18} = 107.3027, P < 0.0001 \)). This is supported by significant interactions between \( pCO_2 \) and time (Fig. 3c; \( F_{1,12.08} = 5.812, P = 0.0268 \)) and \( pCO_2 \) and treatment (GG and untreated) (Fig. 3c; \( F_{1,28.99} = 5.812, P = 0.0268 \)).

**Effect of increasing \( pCO_2 \) on iodine accumulation of seaweeds using in situ mesocosm experiments**

In the mesocosm experiment, elevated \( pCO_2 \) increased tissue growth (relative changes in fresh weight) of all three kelp species compared to ambient \( pCO_2 \) (Fig. 4 a, b and c, \textit{top panels}; \( F_{1,828} = 332.087, P < 0.0001 \)). This effect was consistent over time, which is supported by a significant interaction between time and \( pCO_2 \) conditions (Fig. 4 a, b and c, \textit{top panels}; \( F_{1,828} = 175.387, P < 0.0001 \)). Changes in relative weight were significantly different between the three species (Fig. 4 a, b and c, \textit{top panels}; \( F_{2,828} = \)
including significant interactions between the species of seaweeds and time (Fig. 4 a, b and c, top panels; $F_{2,828} = 38.434, P < 0.0001$), and significant interactions between $pCO_2$ conditions, time and species of seaweeds (Fig. 4 a, b and c, top panels; $F_{2,828} = 28.354, P < 0.0001$). $M. pyrifera$ showed the largest increase in weight under elevated $pCO_2$ at day 40, compared with $S. japonica$ at day 90 and $U. pinnatifida$ at day 60. $S. japonica$ and $U. pinnatifida$ showed similar changes in weight under ambient and elevated $pCO_2$ until day 30, thereafter $U. pinnatifida$ showed larger increases in weight than $S. japonica$ by day 60 (Fig. 4a).

Iodine accumulation increased in the three seaweeds under elevated $pCO_2$ compared to ambient $pCO_2$ (Fig. 4 a, b and c, bottom panels; $F_{1,353} = 36.362, P < 0.0001$). The highest iodine accumulation was observed at time zero, thereafter relative iodine accumulation declined with time (Fig. 4 a, b and c, bottom panels; $F_{1,353} = 791.782, P < 0.0001$) however, iodine accumulation declined more quickly under ambient $pCO_2$ conditions compared to elevated $pCO_2$. The three seaweeds species responded with different intensities to changes in $pCO_2$ (Fig. 4 a, b and c, bottom panels; $F_{2,353} = 16.322, P < 0.0001$). $S. japonica$ showed the highest iodine accumulation at time zero, both under ambient $pCO_2$ (7.91 mg g$^{-1}$ dw ± 0.13 SE) and elevated $pCO_2$ (7.87 mg g$^{-1}$ dw ± 0.19 SE), but it also had the greatest decline in iodine accumulation over 90 days (total decline of 85% and 73% for ambient and elevated $pCO_2$ respectively). Compared to $S. japonica$, $U. pinnatifida$ and $M. pyrifera$ showed modest iodine accumulation at time zero. Iodine accumulation declined under both ambient and elevated $pCO_2$ by 58% and
26% respectively, over 60 days in *U. pinnatifida* (Fig. 4b) and 63% and 56%, over 40 days in *M. pyrifera* (Fig. 4c). This variation in iodine accumulation over time is supported by a significant interaction between species of seaweeds and time (Fig. 4 a, b and c, bottom panels; $F_{2,353} = 16.553, P < 0.0001$). In addition to kelp species (Fig. S6), we also found similar responses to elevated $p$CO$_2$ in other seaweed species, where both algal biomass and iodine accumulation increased with the elevated $p$CO$_2$ (Fig. S7).

**Iodine transfer between *S. japonica* and its consumer *H. discus* in a mesocosm experiment**

In the feeding experiment, abalone fed with *S. japonica*, cultured under elevated $p$CO$_2$ (1000 µatm) had a higher iodine accumulation than those fed on seaweeds cultured under ambient $p$CO$_2$ (400 µatm) (Fig. 5a, $F_{1,106} = 155.24, P < 0.0001$). In addition, iodine accumulation in the abalone was stimulated when the feeding experiments were conducted under elevated $p$CO$_2$ (1000 µatm) (Fig. 5a, $F_{1,106} = 29.53, P < 0.0001$). Iodine accumulation in all experimental abalone increased over the 30 days of cultivation (Fig. 5a, $F_{1,105} = 1003.48, P < 0.0001$), and abalone fed with seaweeds grown under high $p$CO$_2$ and under elevated $p$CO$_2$ conditions showed the highest amount of iodine accumulation (Fig. 5a; *interaction between diet, time and pCO$_2$, $F_{1,102} = 26.29, P < 0.0001$*). There was no difference in iodine accumulation between feeding experiments conducted in a cage or in the field with no cage (Fig. 5a, $F_{1,103} = 1.31, P = 0.255$).

It was found that both T3 (Fig. 5b; $F_{1,33} = 831.98, P < 0.0001$) and T4 (Fig. 5b; $F_{1,33} =$
hormone concentrations declined when the abalone were fed with *S. japonica* cultured under elevated $p$CO$_2$, regardless of the $p$CO$_2$ conditions of the feeding experiment. Whilst there was a decline in thyroid concentration under elevated $p$CO$_2$, for both T3 ($F_{1,34} = 143.29, P < 0.0001$) and T4 concentrations ($F_{1,34} = 183.36, P < 0.0001$), the largest drop in thyroid concentration occurred when abalone were fed with algae cultured under high $p$CO$_2$ and under elevated $p$CO$_2$ conditions.

Linear regression analysis further demonstrated that dietary inclusion of *S. japonica* with higher iodine content caused an increase in iodine intake into the abalone (Fig. S8a) and resulted in lower concentration of T3 (Fig. S8b) and T4 (Fig. S8c) hormones. Moreover, results also revealed that there were significant negative relationships between iodine concentration and T3 (Fig. S9a) and T4 content (Fig. S9b). Overall, THs concentrations were higher in the field with no cage for both T3 (Fig. 5b; $F_{1,32} = 324.30, P < 0.0001$) and T4 (Fig. 5b; $F_{1,32} = 284.49, P < 0.0001$) hormones. However, THs concentration drop in field conditions when abalone were fed with algae cultured under elevated $p$CO$_2$, and this is supported by a significant interaction between the $p$CO$_2$ level that dietary seaweeds were cultured under and the conditions of the feeding experiment (i.e. caged or not caged), for both T3 concentrations (Fig. 5b; $F_{1,31} = 23.93, P < 0.0001$) and T4 concentrations (Fig. 5b; $F_{1,31} = 47.16, P < 0.0001$).

*In situ* transcriptional or proteomic response of *S. japonica* to elevated $p$CO$_2$

Transcriptome data showed that 11 $vIPO$ (vanadium-dependent iodoperoxidases) and 12 $vBPO$ (vanadium-dependent bromoperoxidases) UniGenes were significantly
differentially expressed between ambient and elevated $p$CO$_2$ of culture environments (Fig. 6a). Among these genes, 8 vIPOs and 8 vBPOs were down-regulated, whereas 3 vIPOs and 4 vBPOs were up-regulated under elevated $p$CO$_2$ in the mesocosms (Table S2, S3). The variation trend was verified for 8 genes by RT-PCR (Fig. S10). In the proteomic data, it was found that four vHPOs proteins were down-regulated at elevated $p$CO$_2$ (Fig. 6b). Therein, the trend of relative expression of two vBPOs (SJ19768 and SJ19850) was consistent with those in the transcriptome. In contrast, vIPOs (SJ00628) and vBPOs (SJ10798) did not significantly change at the transcriptomic level.

**DISCUSSION**

Brown algae are predicted to benefit from elevated $p$CO$_2$ under global change (Enochs et al., 2015; Johnson, Russell, Fabricius, Brownlee, & Hall-Spencer, 2012; Linares et al., 2015; Porzio, Buia, & Hall-Spencer, 2011; Xu et al., 2017) and therefore will thrive in natural $p$CO$_2$ rich environments (Enochs et al., 2015; Johnson, Russell, Fabricius, Brownlee, & Hall-Spencer, 2012; Linares et al., 2015; Porzio, Buia, & Hall-Spencer, 2011). It is supported by data that tissue growth of *S. japonica* (Fig. 2 and Fig. 4a), *U. pinnatifida* (Fig. 4b), and *M. pyrifera* (Fig. 4c), and photosynthesis of *S. japonica* (Fig. S11) all increased under elevated CO$_2$ conditions. However, it was unknown how enhanced growth of kelp, under conditions of ocean acidification, affect the accumulation and transfer of iodine across coastal marine foodwebs. Increasing $p$CO$_2$ will not change the solubility and availability of inorganic iodine in seawater (Fig. S5). However, the data presented here reveal for the first time that increasing $p$CO$_2$ causes
iodine accumulation in coastal seaweeds and leads to the accumulation of iodine in consumers of kelp, which potentially will result in elevated iodine intake of consumers of kelp if $pCO_2$ continues to rise (1,000 µatm in 2100 under RCP 8.5) as predicted by climate models (Fig. 1).

In laboratory experiment, we found that at $pCO_2$ greater than 400 µatm (the current $pCO_2$ value), changes in temperature had no effect on iodine accumulation and predictions of iodine accumulation in *S. japonica* could be made based on changes in $pCO_2$ alone (Fig. 2). It is suggested that this is because $pCO_2$ is the dominant environmental driver when $pCO_2$ and temperature change simultaneously. This finding is supported by Brennan and Collins (2015) who demonstrated that changes in the growth rate of the microalgae *Chlamydomonas reinhardtii* could be predicted using the dominant environmental driver in test environments with up to eight environmental drivers (Brennan & Collins, 2015). Global change will involve many simultaneous environmental changes, such as changes in CO$_2$, pH, temperature, and nutrient (Boyd, Lennartz, Glover, & Doney, 2015; Gruber, 2011; Hutchins & Fu, 2017). Results presented here highlight the importance of measuring the response to multiple environmental drivers in order to understand how they interact. Predictions on the combined effects of environmental drivers using traditional additive or multiplicative models could potentially overestimate the effects of ocean warming and ocean acidification on iodine accumulation (Fig. S12).
Elevated iodine accumulation in *S. japonica* under ocean acidification was associated with down-expression regulation of vHPOs (vIPOs and vBPOs), which has never been observed before in any alga (Fig. 6). While vHPOs specific physiological role is still unclear, these genes have previously been studied for their role in the oxidative stress response in brown algae including, *S. japonica* (Ye et al., 2015) and *Laminaria digitata* (Cosse et al., 2009). In addition, the physiological antioxidant role of iodine in algae is suggested to be linked to the presence of particular vHPOs (Colin et al., 2003, 2005).

Under oxidative stress, induced by elicitor-triggered oxidative burst, iodine efflux increased in *S. japonica*, even under high $pCO_2$ (Fig. 3), supporting the physiological role of iodine in algae. In some cases, the iodine efflux was up to twice as high as in the control group (Fig. 3a). Thus, iodine efflux is probably an efficient strategy to cope with oxidative stress (Küpper et al., 2008). Furthermore, the oligoalginate-triggered oxidative burst and associated iodide release is also known to be an efficient strategy against infection, suggesting that under ocean acidification kelp may be able to cope better with infections as they have more stored iodine (Küpper et al., 2008; Küpper, Müller, Peters, Kloareg, & Potin, 2002).

Elevated iodine content in kelp has the potential to be transferred to their consumers such as abalone. *H. discus* fed with algae cultured under elevated $CO_2$ showed higher levels of iodine accumulations and reduced levels of T3 and T4 hormones compared to animals fed with algae cultured under ambient $CO_2$ (Fig. 5). Iodine is essential for the synthesis of thyroid hormones (THs), which play important roles in development,
metamorphosis and metabolism of vertebrates and invertebrates, including humans (Bath et al., 2017; Huang et al., 2015). Previous studies have demonstrated that sea urchin larvae could potentially receive TH precursors or the active hormones from some of the microalgal species that they consumed (Heyland & Moroz, 2006). Whilst, few data were available on the effect of iodine intake on the THs of invertebrates, some studies on humans demonstrate that high-dose kelp supplementation significantly decreased the total triiodothyronine levels of subjects (Eliason, 1998). The increased iodine transfer and reduced thyroid hormones could potentially impact nutrient composition of coastal primary producers as well as in kelp-based coastal food webs.

Our results suggest that projected $p$CO$_2$ (1,000 µatm in 2100 under RCP 8.5) will increase the growth of coastal seaweeds and their iodine accumulation, with the potential for higher levels of iodine to be transferred from seaweeds to consumers. Thus, there is a potential risk of iodine overconsumption in consumers of kelps if $p$CO$_2$ increases as projected for the coming decades. Furthermore, biogeochemical cycling of iodine in coastal ecosystems might change as a consequence of enhanced accumulation in coastal marine foodwebs. This may even have consequences for coastal non-marine habitats as volatile iodinated compounds can be released to the atmosphere (Cosse, Potin, & Leblanc, 2009; Küpper & Kroneck, 2014).

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REFERENCES


**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Carbonate system data (mean ± standard errors) in the laboratory set up using the CO2SYS Package.

**Table S2.** The genes used for real-time quantitative PCR (RT-qPCR) *S. japonica* cultured in field experiment under ambient (bubbled with air) and elevated pCO$_2$ (bubbled with mix air of 1,000 μatm CO$_2$).

**Table S3.** The primers used for real-time quantitative PCR (RT-qPCR) of *S. japonica* cultured in field experiment under ambient (bubbled with air) and elevated pCO$_2$ (bubbled with mix air of 1,000 μatm CO$_2$).

**Fig. S1.** Kinetics of iodine influx by *S. japonica* sporophyte under of various concentrations of KI. (a) Iodine influx rate (IIR) of *S. japonica* sporophyte. (b) The Lineweaver-Burk plots between KI substrate and iodine influx rate which was used for
Lineweaver-Burk $K_m$ and IUR$_{\text{max}}$ determination. In a laboratory set up, kinetics of iodine influx of *S. japonica* under a series of iodide concentration in surrounding medium were determined over a duration of 24 hours. With increasing concentration of iodide (1-30 μmol L$^{-1}$ KI), the influx rate increased significantly until the external KI concentration exceeded 10 μmol L$^{-1}$ (One-Way ANOVA, df=5, $P<0.001$). Furthermore, iodine influx followed a Michaelis-Menten equation and Lineweaver-Burk plot was used for $K_{1/2}$ (19.94 μmol L$^{-1}$) and IUR$_{\text{max}}$ (15.04 μmol g$^{-1}$ dry weight h$^{-1}$) determination ($R^2=0.9547$, df=1, $F=84.229$, $P<0.05$).

**Fig. S2. Experimental design of the field experiment.** $p$CO$_2$ treatments were carried out in triplicate tanks (n=3 per $p$CO$_2$ level), under ambient $p$CO$_2$ (400 μatm, bubbled with air) and elevated $p$CO$_2$ (bubbled with air/CO$_2$ premixed gas using a CO$_2$ Enrichlor). In the field experiment, we held all seaweed species simultaneously as logistics did not allow for each species to be grown on its own at each $p$CO$_2$ level at sufficient replication.

The letters in the net cages represent experimental algal species used in the mesocosms including *S. japonica* (a), *U. pinnatifida* (b), *M. pyrifera* (c), *G. lemaneifor* (d), *G. chouae* (e), *U. pertusa* (f), and *U. intestinalis* (g).

**Fig. S3. Example for coastal mesocosms on the sea.** (a) photograph of net cages and CO$_2$ Enrichlor used for ocean acidification experiment; (b) Variation of nutrient (mean ± standard errors, n=6); (c) Variation in temperature; (d) Variation of irradiance. During the culture period (160 days), the seawater temperature initially decreased from 10.36 ± 0.44 °C to 2.08 ± 0.17 °C after 80 days and then increased from 2.37 ± 0.48 °C to 12.73 ± 0.22 °C. The average irradiance was 98 μmol photons m$^{-2}$ s$^{-1}$ and the light...
condition would not limit algal growth.

**Fig. S4.** Variation of seawater carbonate chemistry (mean ± standard error) under low (400 μatm bubbled with air) or high $p$CO$_2$ (1,000 μatm bubbled with air/CO$_2$ premixed gas using a CO$_2$ Enrichlor) conditions for more than 5 months in the coastal field experiment. (a) pH; (b) Total alkalinity (TA); (c) DIC; (d)HCO$_3^-$; (e) CO$_3^{2-}$; (f) CO$_2$. The shaded areas indicate the standard deviation of three replicates.

**Fig. S5.** The individual and combined effect of increasing $p$CO$_2$ and temperature on iodine solubility and availability. Colored columns show the observed variation of iodide (a) or iodate (b) concentration of three biological replicates (± SE) under increasing $p$CO$_2$ at different temperatures of 5°C, 10°C, 15°C, 20°C and 25°C.

**Fig. S6.** Algal fresh weight (top panels) and iodine accumulation (bottom panels) under ambient $p$CO$_2$ (400 μatm, bubbled with air, blue) and elevated $p$CO$_2$ (bubbled with mix air of 1,000 μatm CO$_2$, red) for (a) *S. japonica*, (b) *U. pinnatifida*, and (c) *M. pyrifera* in mesocosm experiments. Colored circles show the average of biological replicates at each time point (± SE), with n=30 for fresh weight and n=18 for iodine accumulation.

**Fig. S7.** Algal fresh weight (top panels) and iodine accumulation (bottom panels) under ambient $p$CO$_2$ (400 μatm, bubbled with air, blue) and elevated $p$CO$_2$ (bubbled with mix air of 1,000 μatm CO$_2$, red) for (a) *U. pertusa*, (b) *U. intestinalis*, (c) *G. lemaneiformis*, and (d) *G. chouae* in mesocosm experiment. Colored circles show the average of biological replicates at each time (± SE), with n=30 for fresh weight and n=18 for iodine accumulation.
Fig. S8. Linear regression analysis between the iodine concentration of *S. japonica* and iodine concentration or THs synthesis in the consumer *H. discus*. (a) Linear regression analysis between the iodine concentration in *S. japonica* and iodine concentration in *H. discus*; (b) Linear regression analysis between the iodine concentration in *S. japonica* and T3 concentration in *H. discus*; (c) Linear regression analysis between the iodine concentration in *S. japonica* and T4 concentration in *H. discus*.

Fig. S9. Linear regression analysis between the iodine concentration and THs synthesis in *H. discus*. (a) Linear regression analysis between the iodine concentration and T3 concentration; (b) Linear regression analysis between the iodine concentration and T4 concentration.

Fig. S10. Relative expression level of several vHPO genes by real-time quantitative PCR (RT-qPCR).

Fig. S11. The individual and combined effects of temperature and pCO$_2$ on F$_v$/F$_m$ of *S. japonica* in laboratory setup. (a) The individual effect of increasing temperature on F$_v$/F$_m$ where culture pCO$_2$ was set at 400 μatm and bubbled with air; (b) The individual effect of increasing pCO$_2$ on F$_v$/F$_m$ where the temperature was maintained at 10 °C; (c) The combined effects of high temperature of 15 °C and increasing pCO$_2$ on F$_v$/F$_m$; (d) The combined effects of high temperature of 20 °C and increasing pCO$_2$ on F$_v$/F$_m$.

Fig. S12. Comparing the observed effect and the predicted effect of increasing pCO$_2$ and temperature on the kelp *S. japonica*. Filled circles show the
observed response in total iodine accumulation of three biological replicates (± SE) under increasing \( p\text{CO}_2 \) at different temperatures; 10°C (grey), 15°C (yellow) and 20°C (blue). Colored solid lines show the predicted response of iodine accumulation under increasing \( p\text{CO}_2 \) at different temperatures; 15°C (yellow) and 20°C (blue) using (a) the additive model and (b) the multiplicative model. The dashed grey line shows the observed response of increasing \( p\text{CO}_2 \) under control temperature conditions (10°C). This is equivalent to predictions based on the dominant environmental driver (in this example the dominant driver is \( p\text{CO}_2 \)) and demonstrates that predictions on iodine accumulation in *S. japonica* are most accurate when based on the response to \( p\text{CO}_2 \) alone. Note that the y-axis differ between panels a and b.
Fig. 1. Conceptual diagram showing altered iodine metabolic pathway and global iodine geochemical cycle under ocean acidification. The yellow up arrows indicate the up-regulated pathway; the yellow down arrows indicate the down-regulated pathway; the black arrow with dashed lines indicates the possible outcome under further ocean acidification. The abbreviations ROS represents the reactive oxygen species; vHPOs represents the vanadium-dependent haloperoxidases; THs represents the thyroid hormones.
Fig. 2. The combined effect of increasing $p$CO$_2$ and temperature and the effect of increasing temperature alone on iodine accumulation in the kelp *S. japonica*. Filled circles show the average iodide (top panels) and total iodine (bottom panels) accumulation in three biological replicates ($\pm$ SE). (a) Colored circles show the combined effects of increasing $p$CO$_2$ at different temperatures; 10°C (red), 15°C (blue) and 20°C (green). Horizontal dashed lines indicate the iodine accumulation under control temperature (10°C) and $p$CO$_2$ (400 μatm) conditions. (b) Colored circles (orange) show that iodide and total iodine accumulation increases with increasing temperature under control $p$CO$_2$ levels (400 μatm).
Fig. 3. The effect of increasing \( p\text{CO}_2 \) on iodine efflux of \( S. \text{japonica} \) upon oligoguluronate-triggered oxidative burst in laboratory experiment. Colored circles show the average iodine efflux of three biological replicates (± SE) under oligoguluronate elicitor (GG, blue) and Control (red) conditions. (a) The change in iodine efflux of \( S. \text{japonica} \) under GG and control conditions over 5 hours at 10°C; (b) The effect of increasing \( p\text{CO}_2 \) on iodine efflux of \( S. \text{japonica} \) after 3 hours of GG elicitation at 10°C; (c) The short-term elevated \( p\text{CO}_2 \) vs long-term elevated \( p\text{CO}_2 \) effect on iodine efflux 10°C. Treatments shown in the x-axis denote the culture conditions of \( S. \text{japonica}; \) SAC = short-term (7 days) under ambient carbon (400 μatm), SEC = short-term (7 days) under elevated-carbon (1,000 μatm), LAC = long-term (30 days) under ambient-carbon (400 μatm), and LEC = long-term (30 days) under elevated-carbon (1,000 μatm).
Fig. 4. Relative changes in weight (top panels) and relative changes in iodine accumulation (bottom panels) under ambient pCO$_2$ (400 µatm, bubbled with air, blue) and elevated pCO$_2$ (bubbled with mix air of 1,000 µatm CO$_2$, red), in a mesocosm experiment. Three kelp species (a) S. japonica, (b) U. pinnatifida, (c) M. pyrifera were cultured in mesocosm experiment. Colored circles show the average of biological replicates at each time (± SE), with n=30 for the relative changes in weight and n=18 for the relative changes in iodine.
Fig. 5. Changes in iodine accumulation and thyroid hormones (THs) synthesis in *H. discus* fed with seaweeds cultured under either ambient and elevated \(p\text{CO}_2\).

Colored symbols show the average iodine accumulation of six biological replicates (± SE) fed with either *S. japonica* cultured under 400 µatm (circles) or 1,000 µatm (triangles) \(p\text{CO}_2\). Feeding trials were conducted inside a cage under either 1,000 µatm (red symbols) or 400 µatm (blue symbols) \(p\text{CO}_2\) or in the field with no cage under 400 µatm (gray symbols) \(p\text{CO}_2\). (a) Changes in iodine accumulation in *H. discus* were measured every 10 days for 30 days. (b) Changes in the concentrations of triiodothyronine (T3) and thyroxine (T4) at day 30, relative to data collected from feeding experiments with algae cultured under 400 µatm, in the field.
Fig. 6. The effect of increasing $p\text{CO}_2$ on the relative expression of vHPO at transcriptomic or proteomic level in *S. japonica* cultured under ambient (400 μatm) or elevated (1,000 μatm) $p\text{CO}_2$ scenarios, using an *in situ* mesocosm experiment. (a) Variation of vHPO gene expression; (b) Variation of vHPO protein expression. * (p-value <0.05) and ** (p-value <0.001) represent significant differences between treatments of ambient (400 μatm) or high (1,000 μatm) $p\text{CO}_2$. 