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Laminaria kelps impact iodine speciation chemistry in coastal seawater

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Abstract

Kelp (*Laminaria digitata*) uses iodide as a unique inorganic antioxidant to protect its surface and apoplastic space against reactive oxygen species such as ozone, hydrogen peroxide and superoxide, with implications for atmospheric and marine chemistry as well as regional climatic processes. If kelp is covered by seawater, this results in iodide leaching into surrounding sea water. In this study, the influence of the kelps *Laminaria digitata*, *L. hyperborea*, *L. ochroleuca* and *Saccharina latissima* on iodine speciation chemistry was explored at two sites in Oban (Argyll, Scotland) and Roscoff (Brittany, France) based on diver-operated *in situ* sampling. Seawater samples were subsequently analysed voltammetrically, accompanied by determination of extractable iodine concentrations in the tissues of the thalli surveyed by ICP-MS. The main result is that iodide concentrations in the vicinity of kelp thalli are strongly enhanced, especially at low tide, while iodate concentrations are decreased in comparison to open coastal water and open ocean concentrations.

Key words

algae –- iodate- iodide- kelp- halogens – seawater

Abbreviations

cathodic stripping square wave voltammetry (CSSWV)

inductively coupled plasma-mass spectrometry (ICP-MS)

differential pulse voltammetry (DPV)

Dedication

This paper is dedicated to Bernard (Bernez) Kloareg in Roscoff, a great visionary and grandson of a Breton seaweed harvester collecting *Laminaria digitata* for producing iodine, for having inspired and led 4 decades of research on this fascinating model organism and for having built the largest group of scientists in the world focusing on the biochemistry and molecular biology of seaweeds – and for being one of the fathers of the ASSEMBLE network, which has enabled this group to convene to conduct this work.

1. Introduction

The brown alga *Laminaria digitata* is the strongest accumulator of iodine among all living organisms (Ar Gall et al., 2004; Küpper et al., 2008; Küpper et al., 1998). It was recently discovered that this species of kelp (Order Laminariales) accumulates iodide as a unique inorganic antioxidant in its apoplast in order to protect its surface thalli against several aqueous and gaseous oxidants (Küpper et al., 2008). Upon reaction with ozone, volatile molecular iodine is released, resulting in aerosol formation and impacting atmospheric processes (Küpper et al., 2008; Palmer et al., 2005). While iodide is the most suitable halide against most reactive oxygen species (ROS; Küpper et al., 2008), bromide complements iodide for detoxifying superoxide (Küpper et al., 2013). An extensive review of the subject of iodine metabolism in seaweeds (Küpper, 2015; Küpper and Carrano, 2019; Küpper et al., 2011; Küpper and Kroneck, 2015) suggests that this iodide antioxidant system appears to be widespread among other brown and red algae, with its key features (namely, the presence of a haloperoxidase gene in the genome, together with the accumulation of iodide) detected in the brown algal genome model for *Ectocarpus siliculous* (Küpper et al., 2018) and the world's largest seaweed, the giant kelp *Macrocystis pyrifera* (giant kelp; Tymon et al., 2017), suggesting that this is a typical feature of brown algal physiology and biochemistry.

Besides scavenging atmospheric oxidants such as ozone, resulting in the release of molecular iodine to the marine boundary layer of the coastal atmosphere which generates iodine oxides resulting in particle (aerosol) formation (Küpper et al., 2008; Palmer et al., 2005), the antioxidant function of iodide is characterized by its efflux into the surrounding seawater environment (Chance et al., 2009; Küpper et al., 2008; Küpper et al., 1998). Truesdale et al. (2008) observed that the presence of both *Laminaria digitata* and *Fucus serratus* increase iodide *concentrations*, and that *L. digitata* also decreases iodate *concentrations* when grown within seawater aquaria. This is also reflected in the iodide / iodate speciation of the seawater surrounding forests of the giant kelp *Macrocystis pyrifera* near southern California, USA (Gonzales et al., 2017). When diver-operated syringes are used to sample the seawater directly adjacent to the *M. pyrifera* surface thalli, the effects observed are even stronger than when based on coarser-scale, boat-based sampling using Niskin bottles (Carrano et al., 2020), i.e. all samples collected within centimetres of kelp thalli showed reduced levels of total iodine, iodide and iodate, with iodate levels being by far the most affected. Still it is unknown whether *Laminaria* forests which are broadly distributed along the rocky infralittoral of the cold-temperate and Arctic North Atlantic (Bartsch et al., 2008) have comparable effects on iodine speciation in coastal seawater.

In order to address this important knowledge gap, we conducted this study based upon two diving-based sampling campaigns near Oban (Argyll, NW Scotland) and Roscoff (Brittany, France) using large syringes to sample seawater *in situ* from the surface of the thalli of four European kelp species, *L. digitata*, *L. hyperborea*, *L. ochroleuca* and *Saccharina latissima*.

2. Materials and Methods

2.1 Field sampling

Diving-based seawater sampling was conducted near Dunstaffnage, Oban, Scotland, from 4-6 September 2019 inclusive, and near Roscoff, France, from 28 October – 6 November 2019 inclusive (see Tables 1 and 222; Fig. 1).). SamplingSnear RRoscoff was done both bySCUBA within tidal pools containing kelp forests that were partly exposed at low tide (Chenal de l'Ile de Batz), in the shallow infralittoral (Sainte Barbe) and at an offshore site (Astan) using boat-based bottle sampling . Specifically, precision sampling of water at the surface of kelp plants was done at two sites in Oban by SCUBA divers as described recently (Carrano et al., 2020), i.e. by filling two or three syringes (50 ml each, with lead weights attached to each syringe by adhesive tape; Fig. 2) with seawater collected as close as possible (within a centimetre or two) from the kelp holdfast, from within a dense pack of phylloids (i.e. blades), and as a control, at the sea surface more than 10 m away from any kelp plants or the kelp holdfast and phylloid sampling site

In parallel, the phylloid (blade) tissues of the thalli from the proximity of which syringe samples had been collected were also sampled and preserved for determining extractable iodine concentrations. Immediately after collection, all water samples were filtered over 0.45 µm syringe adaptor filters and stored frozen at -20°C until analysis. Seawater temperature data were obtained from a pair of temperature loggers (i.e. averaged from two loggers) deployed at 10 m depth off Dunstaffnage (MDJS, unpublished) and from a dive computer reading on 21 November 2019 in the Baie de Morlaix near Morlaix.

2.2 Iodine speciation analysis in seawater samples

Iodide concentrations were determined directly by cathodic stripping square wave voltammetry (CSSWV) as previously described (Gonzales et al., 2017; Luther III et al., 1988). Iodide concentrations were determined (at least in triplicate (technical replicates) for each of the three syringes collected at the various different sites via cathodic stripping square wave voltammetry (CSSWV) as previously described (Gonzales et al., 2017; Luther III et al., 1988) and quantitated by the method of standard additions (4 additions) using known concentrations of potassium iodide. Iodate concentrations in the same samples described above were measured by minor modifications of the differential pulse voltammetry (DPV) method as reported in Carrano et al. (2020). Here 1.3 mL of sample was pipetted into a voltammetric cell and purged for several minutes with nitrogen to remove residual oxygen. System parameters were as follows: initial potential (-650 mV), final potential (-1400 mV), step size (4 mV), pulse width (40 ms), pulse period (200 ms), pulse amplitude (50 mV), and scan rate (20 mV/s). Iodate concentrations were again determined by the method of standard additions (4 additions) using known concentrations of potassium iodate. All analyses were performed on a BASi controlled growth mercury electrode model MF-9058 interfaced with Epsilon software. A platinum wire served as the auxiliary electrode and an Ag/AgCl electrode was used as the reference electrode.

2.3 Statistical tests

Statistical analyses were done using Systat Ver. 12. Prior to testing, data were examined for normality using Shapiro-Wilk tests and by visual examination of the residuals, and for equal variances using Bartlett's tests, which confirmed that all data met the assumptions of parametric statistics. Following this, separate two-way nested ANOVAs, with the different holdfast sample locations as a random factor nested within each tidal height and offshore location, were used to evaluate differences in iodide and iodate concentrations among the tidal heights and the offshore water, and among different locations at Roscoff. For data collected at Oban, a one-way ANOVA was used to evaluate differences in iodate concentrations among sample locations (kelp blades, holdfasts, and the kelp-free water), and a two sample t-test was used to evaluate differences in iodide concentrations between kelp holdfasts and kelp-free water. The amount of variation explained by differences among sample habitats (i.e. tidal height and offshore waters), sample locations within each habitat, and the replicate syringe samples at each location at Roscoff, and by differences between kelp holdfasts and blades, and the kelp-free water at Oban, were determined using variance components analyses according to Graham and Edwards (2001). Tukey's post hoc tests were used to evaluate pairwise differences when the ANOVAs were significant ($p < 0.05$).

2.4 Determination of iodine concentrations in tissues

Iodine was extracted from algal tissues by alkaline leaching with tetramethylammonium hydroxide (TMAH; Sigma Aldrich, USA) and the total extractable iodine concentration was subsequently determined by ICP-MS. Field-collected samples of blade, holdfast and stipe of a given species were lyophilized and ground to a fine powder using a mortar and pestle. Approximately 0.1 g of the powdered algal material was incubated with 2 mL of 25% TMAH for approximately 72 hours at room temperature using procedures similar to those described in the literature (Radlinger and Heumann, 1998), including our own work (Al-Adilah et al., 2020). The sample was centrifuged and the supernatant was decanted and diluted to approximately 0.25% TMAH. For the determination of the total element content an Agilent 8800 Triple Quadrupole ICP-MS (Agilent Technologies, UK) equipped with a Scott-type spray chamber and a MicroMist concentric glass nebulizer (Glass Expansion, West Melbourne, Australia) was used. The sample and skimmer cones were composed of Ni. The ICP-MS/MS was operated in oxygen (MS/MS-mode) mode for different elements. Collision/reaction cell (CRC) gas flow rates for oxygen were 30% (~ 0.3 mL/min). Instrument calibration was performed by employing iodine standards of up to 1000 ng/mL in dilute TMAH containing Te (Tellurium) as internal standard. Skimmed milk powder (BCR-063R) with a certified iodine concentration of 0.018 ± 0.0018 mmol I/kg was used to check calibration and instrument performance.

3. Results

3.1 Roscoff seawater

Overall, this was our most extensive study of coastal seawater iodate and iodide concentrations thus far. When pooled across all sampling locations and tidal heights, the average iodide concentration in and around the kelp forests at Roscoff was 204 ± 11 nM (mean ± SE) (Figs. 2, 3, 4), while average iodate concentrations were 176 ± 9 nM (Figs. 3, 4), and the average total iodine concentration was approximately 400 nM. It should be noted that we only measured inorganic iodine. However concentrations of organoiodine species are expected to be small i.e. <10% (Chance et al., 2014; Chance et al., 2010; Tian and Nicolas, 1995; Truesdale, 1975). Measurements of organoiodine at Dunstaffnage in Oban done previously validate this assumption (Truesdale, 1975) where organoiodine species comprised only 6.8% of the total. Similar results are expected for Roscoff.

When the two iodine species were examined separately, iodide concentrations varied significantly among seawater collected from the two tidal heights and the offshore surface water (ANOVA: $F_{2,15} = 51.869$, $p < 0.001$), which explained approximately 68% of the total variation in iodide among all seawater samples (Fig. 2, 3, 4). Interestingly, iodide concentrations did not differ among seawater collected from the different kelp holdfast locations ($F_{15,29} = 0.972$, $p = 0.505$), which explained only approximately 8% of the variation among all seawater samples. The remaining 24% of variation in iodide occurred among the three replicate syringe samples collected at each of the holdfast location within each tidal height. Together, iodide concentrations near the kelp holdfasts were approximately 1.7 times greater at low tide (283 ± 11 nM, mean ± SE) than they were at high tide (163 ± 8 nM) (Tukey's HSD, $p < 0.001$), and were almost 2.5 times greater at low tide than in the offshore surface water (118 ± 4 nM; Tukey's HSD, $p < 0.001$). Further, iodide concentrations in the kelp forest were 1.4 times greater at high tide than in the offshore surface water, but this difference was just outside of statistical significance (Tukey's HSD, $p = 0.200$). Altogether, our data indicate four important things. First, iodide concentrations in and around the intertidal kelp forests vary with tidal height and between the kelp forests and offshore water. Second, although iodide concentrations vary at very small scales (i.e. in the seawater surrounding each kelp holdfast), this variation is small compared to variation among different tidal conditions or between the kelp forest and offshore water. Third, although some variation in iodide was observed among the samples collected from the different kelp holdfast locations within the kelp forest (Fig. 323), this variation was small compared to variation among replicate samples collected at each holdfast. Thus, iodide appears to follow a scaling pattern where its variation is greatest at small spatial scales and then decreases with increasing spatial scales, which is similar to patterns of kelp abundance along the west coast of North America (Edwards, 2004). Fourth, iodide concentrations in the offshore surface water were typical of coastal waters (i.e. 100-150 nM), but the concentrations in the kelp forest were extremely high, particularly at low tide.

We observed more overall variation in iodate concentrations than iodide concentrations among our samples, especially among seawater samples collected at the different kelp holdfasts and offshore waters, and among replicate syringes collected at each holdfast. As a result, iodate concentration differences among samples collected from the two tidal heights and the offshore surface water were just outside of statistical significance (ANOVA: $F_{2,15} = 1.189$, $p = 0.111$) and which explained only 11% of the total variation in iodate among all samples (Fig. 4). In contrast, 64% of this variation occurred among the three replicate syringe samples collected at each of the holdfast locations within each tidal height, and 25% of the total variation occurred among seawater collected from the different kelp holdfast locations (Fig. 5), the latter of which was not statistically significant ($F_{15,29} = 1.189$, $p = 0.333$). Perhaps more interesting was that, although just outside of statistical significance, the differences between the tidal heights and offshore sampling locations displayed an opposite pattern compared to that observed for iodide. Specially, iodate concentrations near the intertidal kelp holdfasts were approximately 15% lower at low tide (158 ± 17 nM, mean ± SE) than they were at high tide (181 ± 10 nM), and were 23% lower at high tide than they were in the offshore waters (234 ± 9 nM), but again both of these pairwise differences were just outside statistical significance (Tukey's HSD, $p = 0.341$, $p = 0.121$, respectively) (Fig. 4). Furthermore, iodate concentrations were 35% lower at low tide than in the offshore surface water, but this again was not statistically significant (Tukey's HSD, $p = 0.358$). Perhaps more notable, however, is that average iodate values measured within the kelp forest were approximately 185 nM, which is considerably lower than the average values expected for either open ocean (ca. 450 nM) or coastal water (ca. 350 nM) sites away from kelp forests. The offshore surface waters in this study were near (ca. 10 m) the kelp forest and were well below that in coastal waters in general. However, the anomalously low iodate values determined here are similar to those measured by us in the Point Loma *Macrocystis pyrifera* kelp forest near San Diego (California, USA), where we observed average iodate concentrations of 99-134 nM within the forest, and of ca. 231 nM at a control site near the forest (Gonzales et al., 2017). Together, our iodate data indicate three important things. First, patterns of iodate concentrations were opposite to those of iodide, with greater concentrations in the offshore water and lower concentrations observed within the kelp forest at low tide. Second, as with iodide, iodate concentration followed a scaling pattern where its variation is greatest at small spatial scales and then decreases with increasing spatial scales. Third, iodate concentrations in the offshore surface water and the kelp forests were low compared to average coastal waters but were similar to those observed in kelp forest in other areas.

3.2 Oban seawater

Since the kelp forest in Oban is much less extensive than in Roscoff and is subject to less tidal influence, we focused our attention on measuring iodide and iodate concentrations from samples collected by divers using syringes from the seawater surrounding the kelp forest, and from locations as close as possible to the kelp thalli themselves, specifically the blades and holdfasts. This procedure is similar to a recent but less extensive study of ours (Carrano et al., 2020). Overall, iodate concentrations varied significantly among the three sampling locations (ANOVA: $F_{2,23}$, = 46.99, $p < 0.001$), which explained 85% of the total variation among all samples. The remaining 15% of this variation occurred among replicate samples within each location. Specifically, iodate concentrations in the seawater surrounding the kelp forest were 141 ± 5 nM (mean ± SE) and were significantly greater than those observed at the surface of the kelp blades (61± 6 nM; Tukey's HSD, $p < 0.001$) or at the kelp holdfasts (58 ± 8 nM; Tukey's HSD, $p < 0.001$) (Fig. 6). In contrast, seawater iodate concentrations did not differ between the surface of the kelp blades and at their holdfasts (Tukey's HSD, $p = 0.927$). Thus, while the iodate concentrations measured in the water near the kelp forest were less than that expected for either the open ocean (ca. 450 nM) or coastal waters (ca 350 nM), they were more than twice those measured directly at the kelp blades or holdfasts. Further, they were similar to those measured in the seawater surrounding the kelp forest at Roscoff, and they were comparable to values obtained at the kelp forest located at Point Loma (San Diego CA, USA), where the control sites, blades and holdfasts all gave higher iodate values but which also showed a dramatic reduction of iodate within the holdfasts (from >400 in offshore surface water to <150 nM at the holdfasts). Due to technical difficulties, we were unable to measure iodide concentration near the kelp blades, but values obtained from the seawater near the kelp forest of 155 ± 21 nM and directly from the kelp holdfasts of 197 ± 18 nM did not differ significantly from each other (t-test: $t_{13} = 1.51$, $p = 0.155$), and they were similar to values obtained in Roscoff.

3.3 Tissue iodine concentrations Roscoff and Oban

Iodine recovery of the CRM was (104.3 ± 2.1) % of the certified value. The CRM on milk basis was used, since no algal or other plant-based certified reference material with certified iodine concentrations was available. The detection limit for iodine in seaweed was 0.23 µmol / kg DW.

Details of tissue iodine *concentrations* of all samples are presented in Table 1.

The iodine concentrations in the tissues of *L. digitata* at Roscoff were 34.7±11.5 mmol/kg DW (n=3, in the blades), 30.7±4.84 mmol/kg DW (n=3, in the holdfasts), and 35.9±7.25 mmol/kg DW (n=3, in the stipes). At Oban, the iodine concentrations in the tissues of *L. digitata* were 41.3±8.81 mmol/kg DW (n=3, in the blades), while that of the holdfast was 25.8±6.63 mmol/kg DW (n=3, in the holdfasts), and 64.9±14.4 mmol/kg DW (n=3, in the stipes). The iodine concentrations in the tissues of *L. hyperborea* at Roscoff were 24.6 mmol/kg DW (in the blades), 28.8 mmol/kg DW (in the holdfasts), and 19.3 mmol/kg DW (in the stipes). The iodine concentrations in the tissues of *Saccharina latissima* at Oban were 46.3 mmol/kg DW in the blades, 17.7 mmol/kg DW in the holdfasts, and 86.5 mmol/kg DW in the stipes. At Oban, the iodine concentrations in the tissues of *S. latissima* were 23.2 mmol/kg DW (in the blades), while that of the holdfast was 34.2 mmol/kg DW (in the holdfasts), and 13.1 mmol/kg DW (n the stipes). The stipe concentration in *Laminaria ochroleuca* was 35.8 mmol/kg DW.

4. Discussion

Adding these results to those obtained in two previous studies of ours (Carrano et al., 2020; Gonzales et al., 2017) and to the aquarium experiments by Truesdale et al. (2008), several things stand out. First that under conditions resulting in oxidative stress i.e. low tide, high temperatures etc., iodide concentrations in the surrounding seawater are considerably elevated from the values expected for either, open ocean or kelp free coastal waters. Indeed, under oxidative stress conditions, iodide concentrations are actually higher than those of iodate in stark contrast to the situation in kelp-free areas. This pattern is largely maintained irrespective of the specific brown algal kelp species present (*Macrocystis vs Laminaria*) or geographical location (Pt. Loma, San Diego, California, vs Oban, Scotland, UK, or Roscoff, Brittany, France). However, the effect is most obvious for the low tide samples from Roscoff where the tidal effects are large and leave large swaths of the *Laminaria* kelp bed uncovered and subjected to extreme desiccation and oxidative stress. The effect is much more modest in Oban and Pt. Loma where the kelp is never fully exposed to the atmosphere even at the lowest tides and thus these kelp experience far less oxidative stress. This effect is largely as expected as it has been known for a long time that th kelps release iodide (presumably as an antioxidant) under oxidative stress conditions (Ar Gall et al., 2004; Küpper et al., 2008; Küpper et al., 1998). What is novel here is that we now show that this effect is large enough to change both the speciation and the total iodine concentrations in the surrounding seawater dramatically. Of course iodine concentrations and speciation are not driven only by macroalgae such as those studied here. Blooms of phytoplankton are also common in coastal regions and increasing evidence suggest that they also contribute to increasing iodide values in coastal environments likely caused by cell senescence particularly in bloom declines (Bluhm et al., 2010; Carrano et al., 2020). However in this case increased iodide results in a concomitant decrease in iodate as there is mostly just a change in speciation rather than in total iodine present in the seawater, i.e. there must be a strong reducing force acting upon the iodate present.

The second major conclusion from our study is that iodate concentrations in and around the kelp forests are dramatically lower than expected for coastal waters. Further, there is not a simple 1:1 correspondence between decreased iodate and increased iodide, suggesting that the effect is not due to simple changes in speciation caused by reduction (by an unknown source) of iodate to iodide but is rather due to a removal of iodine, as iodate, from the seawater. This is consistent with our earlier field study in the Pt. Loma kelp beds (Gonzales et al., 2017) which showed a statistically significant loss of iodate without a corresponding increase in iodide in the middle of the *M. pyrifera* beds during the summer months when kelp biomass was at its maximum, and with laboratory experiments with the brown alga *Ectocarpus siliculosus* which also showed long term decrease in iodate values (Carrano et al., 2020). The simplest hypothesis to account for these observations is that iodate itself is being actively taken up as originally proposed by Truesdale (2008). However, a number of alternative theories to account for the loss of iodate without a concomitant increase in iodide exist. Indeed, we have proposed that iodate is not taken up directly but rather is reduced to iodide at the surface of macroalgal cells by cell surface reductases and then transported into such cells as iodide without re-entering the bulk solution. Such NAD(P)H dependent cell surface reductases are known to exist in macroalgae (Böttger et al. 2012; Cock et al. 2010) and to have redox potentials capable of reducing iodate to iodide. Our results here are entirely in agreement with such a model.

Given the limited data there was no statistically significant difference in tissue iodine *concentrations* observed based on kelp species, location or tissue type. Overall tissue iodine *concentrations* observed here were very much in the range reported before for *L. digitata*, *L. hyperborea* and *S. latissima* (Ar Gall et al., 2004; Küpper et al., 1998; Saenko et al., 1978). As a noteworthy aside, to our knowledge, this study is the first to report an iodine concentration for the Lusitanian kelp *L. ochroleuca*, which is currently extending its range northward along the European Atlantic coast which is thought to be a consequence of climate changes (Küpper and Kamenos, 2018).

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6. References

Al-Adilah, H., Peters, A.F., Al-Bader, D., Raab, A., Akhdhar, A., Feldmann, J., Kupper, F.C., 2020. Iodine and fluorine concentrations in seaweeds of the Arabian Gulf identified by morphology and DNA barcodes. Bot. Mar. 63, 509-519.
Ar Gall, E., Küpper, F.C., Kloareg, B., 2004. A survey of iodine contents in *Laminaria digitata*. Bot. Mar. 47, 30-37.
Bartsch, I., Wiencke, C., Bischof, K., Buchholz, C.M., Buck, B.H., Eggert, A., Feuerpfeil, P., Hanelt, D., Jacobsen, S., Karez, R., Karsten, U., Molis, M., Roleda, M.Y., Schubert, H., Schumann, R., Valentin, K., Weinberger, F., Wiese, J., 2008. The genus *Laminaria* sensu lato: recent insights and developments. European Journal of Phycology 43, 1-86.
Bluhm, K., Croot, P., Wuttig, K., Lochte, K., 2010. Transformation of iodate to iodide in marine phytoplankton driven by cell senescence. Aquatic Biology 11, 1-15.
Carrano, M.W., Yarimizu, K., Gonzales, J.L., Cruz-Lopez, R., Edwards, M.S., Tymon, T.M., Kupper, F.C., Carrano, C.J., 2020. The influence of marine algae on iodine speciation in the coastal ocean. Algae 35, 167-176.
Chance, R., Baker, A.R., Küpper, F.C., Hughes, C., Kloareg, B., Malin, G., 2009. Release and transformations of inorganic iodine by marine macroalgae. Estuarine Coastal and Shelf Science 82, 406-414.
Edwards, M.S., 2004. Estimating scale-dependency in disturbance impacts: El Ninos and giant kelp forests in the northeast Pacific. Oecologia 138, 436-447.
Gonzales, J., Tymon, T., Küpper, F.C., Edwards, M.S., Carrano, C.J., 2017. The potential role of kelp forests on iodine speciation in coastal seawater. PLoS One 12, e0180755.
Küpper, F.C., 2015. Iodine in Seaweed: Two Centuries of Research. Springer-Verlag Berlin, Berlin.
Küpper, F.C., Carpenter, L.J., Leblanc, C., Toyama, C., Uchida, Y., Verhaeghe, E., Maskrey, B., Robinson, J., Boneberg, E.-M., Malin, G., Luther III, G.W., Kroneck, P.M.H., Kloareg, B., Meyer-Klaucke, W., Muramatsu, Y., Potin, P., Megson, I.L., Feiters, M.C., 2013. Speciation studies and antioxidant properties of bromine in *Laminaria digitata* reinforce the significance of iodine accumulation for kelps. J. Exp. Bot. 64, 2653-2664.
Küpper, F.C., Carpenter, L.J., McFiggans, G.B., Palmer, C.J., Waite, T.J., Boneberg, E.M., Woitsch, S., Weiller, M., Abela, R., Grolimund, D., Potin, P., Butler, A., Luther III, G.W., Kroneck, P.M.H., Meyer-Klaucke, W., Feiters, M.C., 2008. Iodide accumulation provides kelp with an inorganic antioxidant impacting atmospheric chemistry. Proceedings of the National Academy of Sciences of the United States of America 105, 6954-6958.
Küpper, F.C., Carrano, C.J., 2019. Key aspects of the iodine metabolism in brown algae: a brief critical review. Metallomics 11, 756-764.
Küpper, F.C., Feiters, M.C., Olofsson, B., Kaiho, T., Yanagida, S., Zimmermann, M.B., Carpenter, L.J., Luther III, G.W., Lu, Z., Jonsson, M., Kloo, L., 2011. Commemorating two centuries of iodine research: An interdisciplinary overview of current research. Angewandte Chemie - International Edition 50, 11598 – 11620.
Küpper, F.C., Kamenos, N.A., 2018. The future of marine biodiversity and marine ecosystem functioning in UK coastal and territorial waters (including UK Overseas Territories) – with an emphasis on marine macrophyte communities. Bot. Mar. 61, 521-535.
Küpper, F.C., Kroneck, P.M.H., 2015. Iodine Bioinorganic Chemistry: Physiology, Structures, and Mechanisms, in: Kaiho, T. (Ed.), Iodine Chemistry and Applications. John Wiley & Sons Inc, Hoboken, pp. 557-589.
Küpper, F.C., Miller, E.P., Andrews, S.J., Hughes, C., Carpenter, L.J., Meyer-Klaucke, W., Toyama, C., Muramatsu, Y., Feiters, M.C., Carrano, C.J., 2018. Emission of volatile halogenated compounds,

speciation and localization of bromine and iodine in the brown algal genome model Ectocarpus siliculosus. Journal of Biological Inorganic Chemistry (JBIC) 23, 1119–1128.
Küpper, F.C., Schweigert, N., Ar Gall, E., Legendre, J.M., Vilter, H., Kloareg, B., 1998. Iodine uptake in Laminariales involves extracellular, haloperoxidase-mediated oxidation of iodide. Planta 207, 163-171.
Luther III, G.W., Swartz, C.B., Ullman, W.J., 1988. Direct Determination of Iodide in Seawater by Cathodic Stripping Square Wave Voltammetry. Anal. Chem. 60, 1721-1724.
Palmer, C.J., Anders, T.L., Carpenter, L.J., Küpper, F.C., McFiggans, G.B., 2005. Iodine and halocarbon response of *Laminaria digitata* to oxidative stress and links to atmospheric new particle production. Environ. Chem. 2, 282-290.
Radlinger, G., Heumann, K.G., 1998. Iodine determination in food samples using inductively coupled plasma isotope dilution mass spectrometry. Anal. Chem. 70, 2221-2224.
Saenko, G.N., Kravtsova, Y.Y., Ivanenko, V.V., Sheludko, S.I., 1978. Concentration of iodine and bromine by plants in the seas of Japan and Okhotsk. Marine Biology 47, 243 - 250.
Truesdale, V.W., 2008. The biogeochemical effect of seaweeds upon close-to natural concentrations of dissolved iodate and iodide in seawater - Preliminary study with *Laminaria digitata* and *Fucus serratus*. Estuarine Coastal and Shelf Science 78, 155-165.
Tymon, T.M., Miller, E.P., Gonzales, J.L., Raab, A., Küpper, F.C., Carrano, C.J., 2017. Some aspects of the iodine metabolism of the giant kelp *Macrocystis pyrifera* (Phaeophyceae). Journal of Inorganic Biochemistry 177, 82-88.

Tables

Table. 1. Details of all field sampling conducted within the framework of this study – and of the total iodine concentrations (determined by ICP-MS) contained in representative thalli collected at Roscoff, Brittany, France..

Table. 2. Details of all field sampling conducted within the framework of this study – and of the total iodine concentrations (determined by ICP-MS) contained in representative thalli collected at Oban, Scotland, UK.

Date / numbers of water samples (collected by syringe)	**Site, GPS coordinates**	**Species**	**Tissue iodine concentration (mmol / kg DW)**	**Water depth**	**Water temperature**	**Topographic and ecological features of the site**
28 October 2019 **1** 281019-1.1 281019-1.2 281019-1.3	Chenal de l'Ile Batz / Ile Verte, Roscoff (2 on map) N48.73297° W003.98705°	*Laminaria ochroleuca*	Stipe 35.4	intertidal, low tide (about 1.5 m)	11.7°C on 21 November	Intertidal, shallow and sheltered from strong wave action, yet high currents between headland and adjacent island
28 October 2019 **2**	Chenal de l'Ile Batz / Ile Verte, Roscoff	*Laminaria digitata*	Holdfast 26.8	intertidal, low tide	11.7°C on 21 November	Intertidal, shallow and sheltered

281019-2.1 281019-2.2 281019-2.3	(2 on map) N48.73297° W003.98705°			(about 1.5 m)		from strong wave action, yet high currents between headland and adjacent island
28 October 2019 **3** 281019-3.1 281019-3.2 281019-3.3	Chenal de l'Ile Batz / Ile Verte, Roscoff (2 on map) N48.73297° W003.98705°	*Laminaria digitata*	Blade 39.3	intertidal, low tide (about 1.5 m)	11.7°C on 21 November	Intertidal, shallow and sheltered from strong wave action, yet high currents between headland and adjacent island
30 October 2019	Sainte Barbe, Roscoff	*Laminaria digitata*	Stipe 51.2	intertidal, low tide	11.7°C on 21 November	Exposed headland

4 301019-1.1 301019-1.2 301019-1.3	(3 on map) N48.72570° W003.96783°			(about 1.5 m)		rapidly dropping off into deeper waters
30 October 2019 **5** 301019-2.1 301019-2.2 301019-2.3	Sainte Barbe, Roscoff (3 on map) N48.72570° W003.96783°	*Laminaria digitata*	Holdfast 27.2	intertidal, low tide (about 1.5 m)	11.7°C on 21 November	Exposed headland rapidly dropping off into deeper waters
30 October 2019 **6** 301019-3.1 301019-3.2 301019-3.3	Sainte Barbe, Roscoff (3 on map) N48.72570° W003.96783°	*Laminaria digitata*		intertidal, low tide (about 1.5 m)	11.7°C on 21 November	Exposed headland rapidly dropping off into deeper waters

4 November 2019 **7** 041119-1.1 041119-1.2 041119-1.3	Sainte Barbe, Roscoff (3 on map) N48.72570° W003.96783°	*Laminaria hyperborea*	Blade 25.1	7 m, high tide	11.7°C on 21 November	Exposed headland rapidly dropping off into deeper waters
4 November 2019 **8** 041119-2.1 041119-2.2 041119-2.3	Sainte Barbe, Roscoff (3 on map) N48.72570° W003.96783°	*Laminaria hyperborea*	Stipe 19.3	7 m, high tide	11.7°C on 21 November	Exposed headland rapidly dropping off into deeper waters
4 November 2019 **9** 041119-3.1 041119-3.2 041119-3.3	Sainte Barbe, Roscoff (3 on map) N48.72570° W003.96783°	*Laminaria hyperborea*	Holdfast 28.8	7 m, high tide	11.7°C on 21 November	Exposed headland rapidly dropping off into deeper waters

6 November 2019 **10** 061119-1.1 061119-1.2 061119-1.3	Sainte Barbe, Roscoff (3 on map) N48.72570° W003.96783°	*Laminaria digitata*	Blade 24.6	6.6 m, high tide	11.7°C on 21 November	Exposed headland rapidly dropping off into deeper waters
6 November 2019 **11** 061119-2.1 061119-2.2 061119-2.3	Sainte Barbe, Roscoff (3 on map) N48.72570° W003.96783°	*Laminaria digitata*	Stipe 17.4	6.6 m, high tide	11.7°C on 21 November	Exposed headland rapidly dropping off into deeper waters
6 November 2019 **12** 061119-3.1 061119-3.2 061119-3.3	Sainte Barbe, Roscoff (3 on map) N48.72570° W003.96783°	*Laminaria digitata*	Holdfast 37.9	6.6 m, high tide	11.7°C on 21 November	Exposed headland rapidly dropping off into deeper waters

6 November 2019 **13** 061119-4.1 061119-4.2 061119-4.3	Chenal de l'Ile Batz / Ile Verte, Roscoff (2 on map) N48.73297° W003.98705°	*Laminaria digitata*		intertidal, low tide (about 1.5 m)	11.7°C on 21 November	Intertidal, shallow and sheltered from strong wave action, yet high currents between headland and adjacent island
6 November 2019 **14** 061119-4.1 061119-4.2 061119-4.3	Chenal de l'Ile Batz / Ile Verte, Roscoff (2 on map) N48.73297° W003.98705°	*Laminaria digitata*		intertidal, low tide (about 1.5 m)	11.7°C on 21 November	Intertidal, shallow and sheltered from strong wave action, yet high currents between headland and

						adjacent island
6 November 2019 **15** 061119-6.1 061119-6.2	Chenal de l'Ile Batz / Ile Verte, Roscoff (2 on map) N48.73297° W003.98705°	*Laminaria digitata*		intertidal, low tide (about 1.5 m)	11.7°C on 21 November	Intertidal, shallow and sheltered from strong wave action, yet high currents between headland and adjacent island
6 November 2019 **16** ctrl 1 ctrl 2 ctrl 3	Astan, Roscoff (1 on map) N48.75310° W003.96000°	Open water (control)		surface layer above 50.8 m water depth	11.7°C on 21 November	Exposed headland rapidly dropping off into deeper waters

Table 2.

Date / numbers of water samples (collected by syringe)	**Site, GPS coordinates**	**Species**	**Tissue iodine concentration (mmol / kg DW)**	**Water depth**	**Water temperature**	**Topographic and ecological features of the site**
4 September 2019	Danger Reef, Oban (1 on map) N56°28'22.1" W005°27'44.5"	*Laminaria hyperborea*	Stipe 86.5 Holdfast 17.7 Blade 46.3	9.8	14.1°C	Deep-reef, fairly exposed
5 September 2019	Saulmore Point, Oban (2 on map) N56°27'16.1" W005°24'49.7"	*Laminaria digitata*	Stipe 59.4 Holdfast 35.8 Blade 28.1	8.1	13.9°C	High-current headland at the mouth of a fjord (Loch Etive)
6 September 2019	Castle Gardens, Oban (3 on map)	*Laminaria digitata*	Stipe 48.9 Holdfast 24.0	7.1	13.9°C	Site with Site with strong

	N56°27'16.9" W005°26'07.2"		Blade 49.6			tidal current between Loch Linnhe and Dunstaffnage Bay
6 September 2019	Eilean Mor, Oban (4 on map) N56°27'22.8" W005°26'46.3"	*Laminaria digitata*	-	6	13.9°C	High current site at small island adjacent to Dunstaffnage Peninsula
6 September 2019	Castle Gardens, Oban (3 on map) N56°27'16.9" W005°26'07.2"	*Laminaria hyperborea*	Stipe 13.1 Holdfast 34.2 Blade 23.2	6	13.9°C	Site with Site with strong tidal current between Loch Linnhe and Dunstaffnage Bay

6 September 2019	Pontoon Bay, Oban (5 on map) N56°27'14.3" W005°26'09.3"	*Saccharina latissima*	Stipe 86.5 Holdfast 17.7 Blade 46.3	6 m	13.9°C	Sheltered site with little current in Dunstaffnage Bay

Tab. 3. Tissue iodine levels reported by other studies.

Species	**Tissue type**	**Concentration (g / kg DW)**	**Reference**
Laminaria japonica	Not specified	5.6	Saenko et al. (1978)
Laminaria digitata	Young thalli (< 15 cm)	47	Fig. 1 in Küpper et al. (1998)
	Adult thalli (3-4 years old)	4	Küpper et al. (1998)
	Blade of a young adult thallus	3.7 – 16.3	Fig. 1 in Küpper et al. (1998)
	Holdfast of young adult thallus (the same as previous line)	5.2 – 19.1	Fig. 1 in Küpper et al. (1998)
	Holdfast of young adult thallus (the same as previous 2 lines)	3.7	Fig. 1 in Küpper et al. (1998)
	Blade of adult thallus (3-4 years old)	1.8 – 5.2	Fig. 1 in Küpper et al. (1998)
Saccharina latissima	Protoplasts	3.3 to 7.4%	Küpper et al. (1998)
Laminaria digitata	Blade of adult thalli	Strong seasonal differences: High iodine concentrations during the cold season (8.5 – 17.5 g / kg DW), lower during the warm season (3.2 – 12.9 g / kg DW). Small thalli have much higher concentrations than older, large thalli.	Ar Gall et al. (2004); especially Table 1 and Fig. 2 therein for a detailed listing of iodine concentrations.

Figure Captions

Figure 1. Maps of the study sites. (A) Roscoff with (1) Astan, (2) Chenal de l'Ile and Batz and (3) Sainte Barbe. (B) Oban with (1) Danger Reef, (2) Saulmore Point, (3) Castle Gardens, (4) Eilean Mor and (5) Pontoon.

Figure 2. Diver (FCK) operating the syringe sampling technique off Dunstaffnage (near Oban, Scotland), which is the basis for this study.

Figure 3. Iodide concentrations in nM (mean ± SE) as determined by cathodic stripping square wave voltammetry (CSSWV) of 16 samples (in triplicate) collected from offshore water (control), and in the kelp forest at low tide and at high tide in Roscoff, Brittany, France between October 28 through November 6, 2019. The horizontal hatched line denotes the average concentration across all samples. Error bars are based on $n = 3$ per sample. Information about the location, date etc. for each sample number are given in Table 1.

Figure 4. Iodide and iodate concentrations in nM (mean ± SE) as determined by cathodic stripping square wave voltammetry CSSWV in seawater collected offshore (control), and in the kelp forest at low tide and high tide. Bars with above them denote statistically significant differences as determined by Tukey's HSD post hoc tests ($p < 0.05$). Capital letters reference iodide and lower case denote iodate. Error bars are based on sample sizes of $n = 6$ for low tide, $n = 9$ for high tide, and $n = 3$ for offshore.

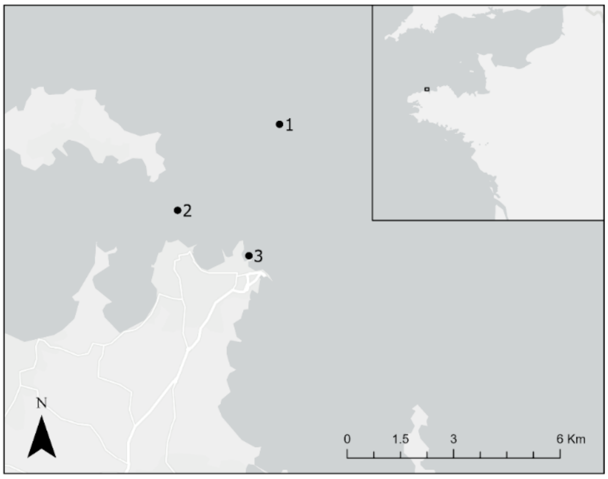
Figure 5. Iodate concentrations in nM (mean ± SE) as determined by differential pulse cathodic stripping voltammetry (DPV) of 16 samples collected from offshore water (control), and in the kelp forest at low tide and at high tide in Roscoff, Brittany, France between October 28 through November 6, 2019. The horizontal line indicates the average value obtained among all samples. The horizontal hatched line denotes the average concentration across all samples. Error bars are based on sample sizes of $n = 6$ for low tide, $n = 9$ for high tide, and $n = 3$ for offshore. Information about the location, date etc. for each sample number are given in Table 1.

Figure 6. Iodide and iodate concentrations nM (mean ± SE) as determined by cathodic stripping square wave voltammetry CSSWV in seawater collected near kelp holdfasts and kelp blades, and in kelp-free seawater in Oban, Scotland, UK September 4-6, 2019. Bars with different letters above them denote statistically significant differences as determined by Tukey's HSD post hoc tests ($p < 0.05$). Capital letters reference iodide and lower case denote

iodate. Concentrations of iodide for samples collected near the blades were not useable due to technical issues, and thus are denoted by “ND”. Error bars are based on n = 8 for kelp-free water, n = 8 for kelp blades, and n = 10 for kelp holdfasts.

Figure 1

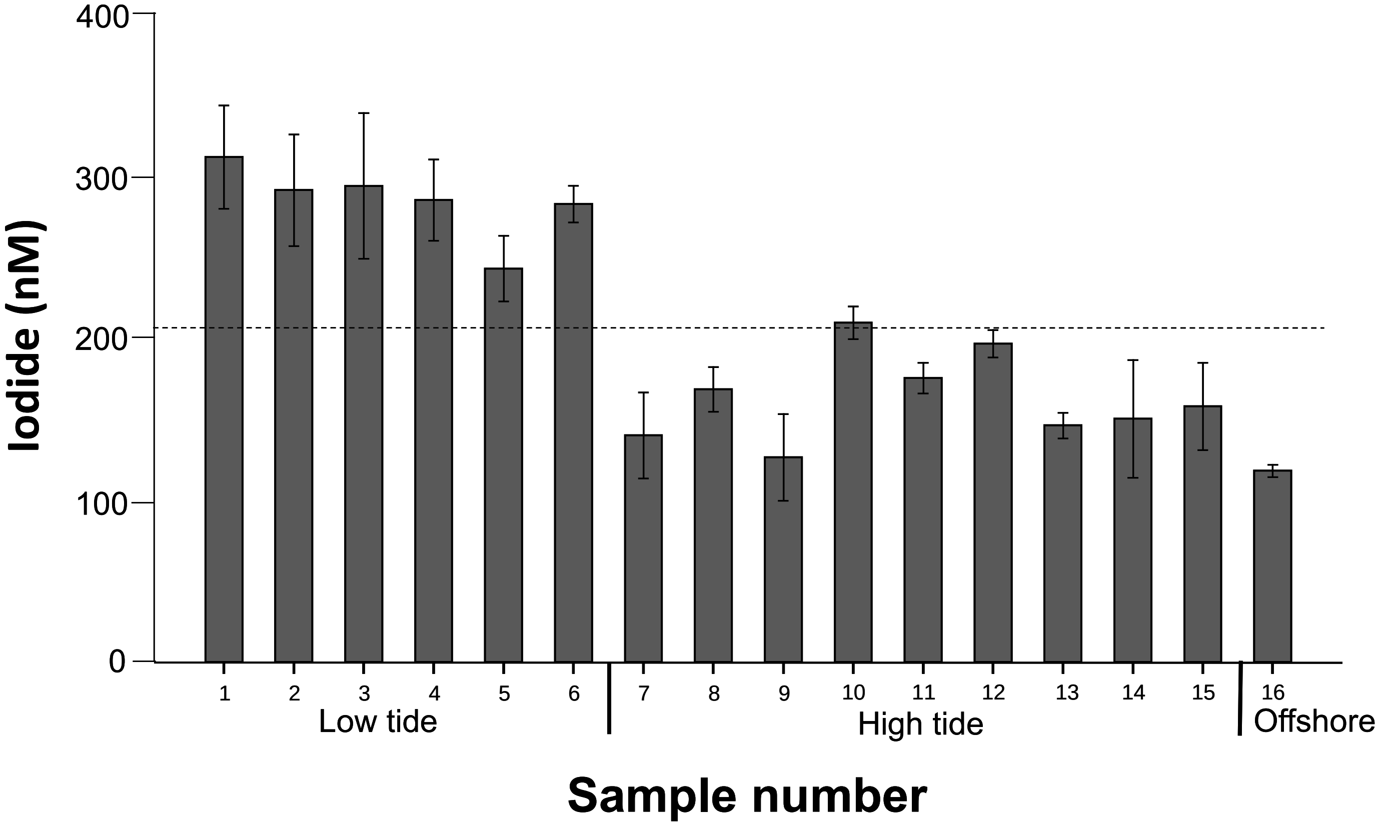
A



B

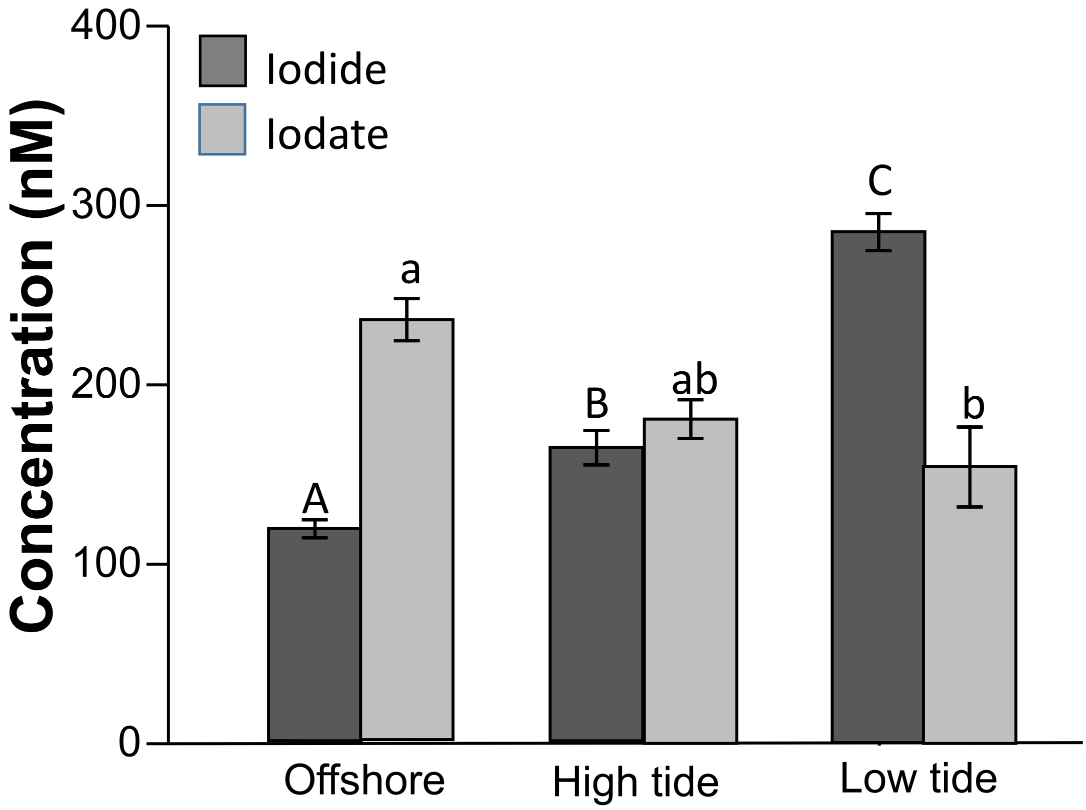
Figure 2

Figure 3



400
300
200
100
0
Iodide (nM)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Low tide
High tide
Offshore
Sample number

Figure 4



Concentration (nM)
400
300
200
100
0
Iodide
Iodate
A
a
B
ab
C
b
Offshore
High tide
Low tide

Figure 5

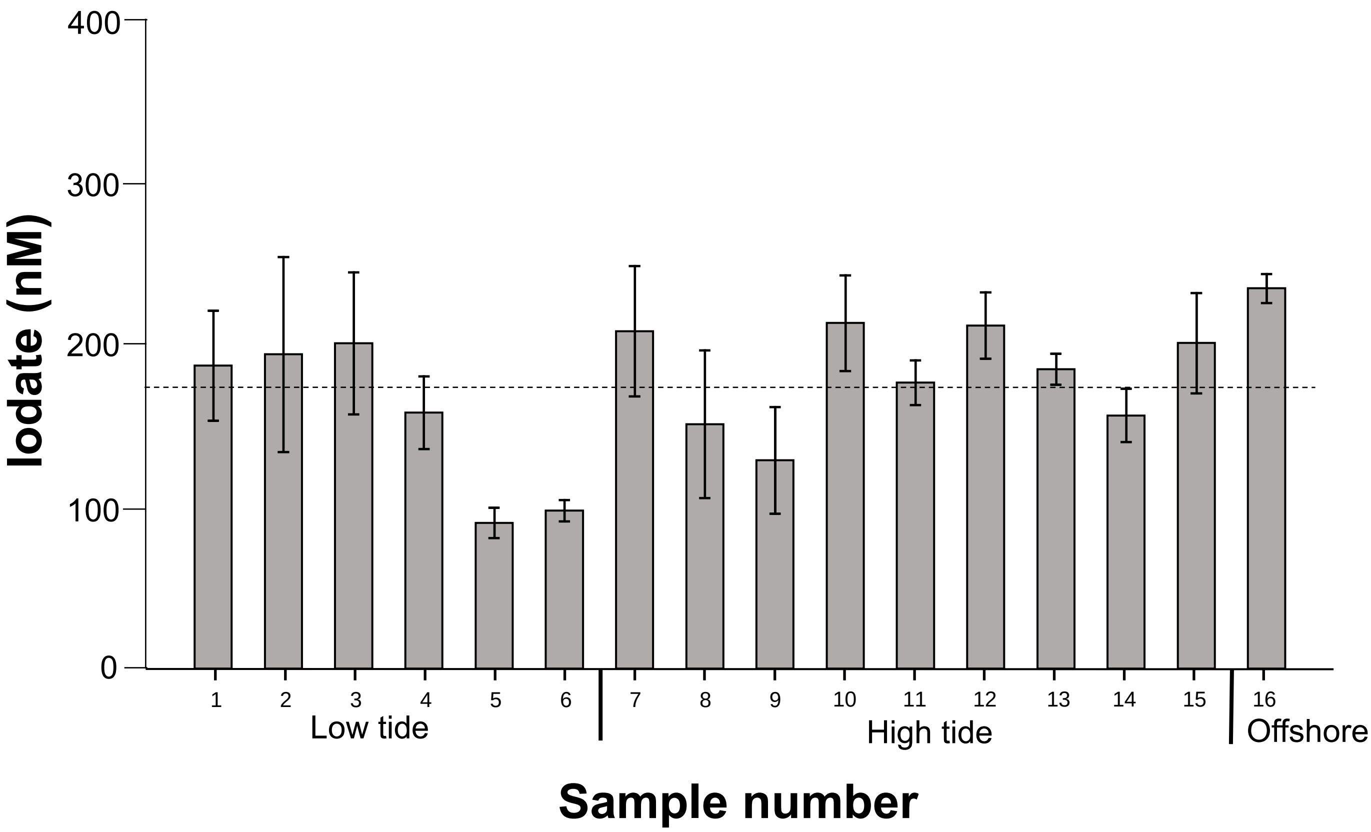
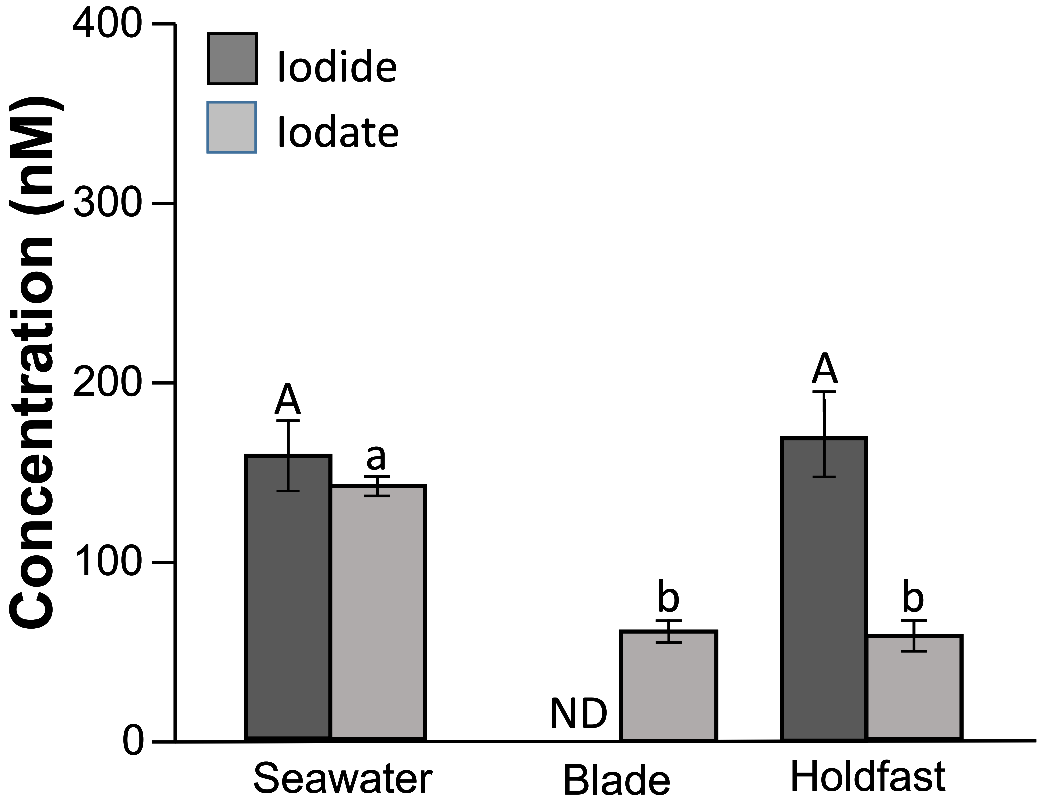

400
300
200
100
0
Iodate (nM)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Low tide
High tide
Offshore
Sample number

Figure 6


Concentration (nM)
400
300
200
100
0
Iodide
Iodate
A
a
A
b
b
ND
Seawater
Blade
Holdfast