Iodine uptake in brown seaweed exposed to radioactive liquid discharges from the reprocessing plant of ORANO La Hague

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FIEVET Bruno[a], VOISEUX Claire[a], LEBLANC Catherine[b], MARO Denis[a], HEBERT Didier[a], SOLIER Luc[a], GODINOT Claire[a]*

[a]Institut de Radioprotection et de Sûreté Nucléaire, PSE-ENV/SRTE, Laboratoire de Radioécologie de Cherbourg-Octeville, France

[b]Sorbonne Université, CNRS, UMR 8227, Integrative Biology of Marine Models, Station Biologique de Roscoff, Roscoff, France

* corresponding author
Claire GODINOT. IRSN, Laboratoire de Radioécologie, Rue Max Pol Fouchet, BP10, 50130 Cherbourg-en-Cotentin, France.
claire.godinot@irsn.fr.

Abstract

Iodine-129 is present in controlled liquid radioactive waste routinely released in seawater by the ORANO nuclear fuel reprocessing plant in La Hague (Normandy, France). Brown algae are known for their exceptional ability to concentrate iodine from seawater. They also potentially emit volatile iodine compounds in response to various stresses, such as during emersion at low tide. For these reasons, brown seaweed is routinely collected for radioactivity monitoring in the marine environment. Despite the high concentration ratio the exact mechanism of iodine uptake is still unclear. Chemical imaging by laser desorption/ionization mass spectrometry provided evidence that iodine is stored by kelps as I^-. In this study we investigate *in vivo* iodine uptake in kelps (*Laminaria digitata*) with an emphasis on seawater iodine chemical speciation. Our results showed that kelp plantlets were able to take up iodine in the forms of both IO_3^- and I^-. We also observed transient net efflux of I^- back to seawater but no IO_3^- efflux. Since the seaweed stores I^- but takes up both IO_3^- and I^-, IO_3^- was likely to be converted into I^- at some point in the plantlet, or through the action of associated microorganisms. One major outcome of our experiments was the direct observation of the kelp-based biogenic conversion of seawater IO_3^- into I^-. On the basis of both IO_3^- and I^- uptakes by the seaweed, we propose new steps in the possible iodine concentration mechanism used by kelp.

Keywords: radioactive iodine, uptake, kelp, chemical speciation

1 Introduction

Though a minor element in seawater, iodine is mainly present on Earth dissolved in the oceans (Carpenter, 2003; Carpenter et al, 2021; Chance et al, 2014; Wong, 1991). In the terrestrial environment, it is present at very low levels in soils, water and plants, but in vertebrates, it is involved in the crucial functions of the thyroid gland as organically bound in thyroid hormones (Fuge & Johnson, 2015). Iodine is also present as multiple volatile compounds involved in a complex geochemical cycle between the oceans and the atmosphere. The high reactivity of iodine is considered to play a key role in atmospheric chemistry and possibly the climate, at least in coastal environments (Carpenter et al, 2021; Carpenter et al, 2013; Kolb, 2002; Saiz-Lopez et al, 2012). In addition to stable ^{127}I, iodine has 36 radioactive isotopes, and with the exception of ^{129}I (half-life of 15.7 10^6 years), most iodine radioisotopes quickly decay (half-lives of a few days). Two main iodine radioisotopes are generated by

human activities. ^{131}I is a short-lived (half-life of 8.5 days) fission by-product of ^{235}U in nuclear electricity production, and high activities are also used in medicine for cancer radiotherapy. ^{129}I is generated in nuclear reactors, and because of its very slow decay rate, it is a major residual iodine radioisotope in the nuclear fuel cycle, which adds to naturally occurring ^{129}I (cosmic ray spallation of Xe in the atmosphere and spontaneous fission of natural uranium). It is also present in fallout from past atmospheric nuclear weapons testing and major powerplant accidents. ^{129}I is present in controlled liquid radioactive waste routinely released in seawater by the ORANO nuclear fuel reprocessing plant in La Hague (Normandy, France) and the annual amounts released are between 1 and 2 $TBq.yr^{-1}$ (Fiévet et al, 2020). Discharging liquid ^{129}I waste into seawater results in isotopic dilution in naturally-present stable ^{127}I. The background isotopic ratio $^{129}I/^{127}I$ is in the range [10^{-13}-10^{-12}] and reaches [10^{-7}-10^{-6}] in seawater in the English Channel (Hou et al, 2007). Considering a concentration of dissolved ^{127}I in seawater of 0.5 µM, this means ^{129}I activities in the range [0.05-0.5] $Bq.m^{-3}$. However, higher seawater ^{129}I activities could be expected in the close vicinity of the ORANO plant outlet.

Brown algae are known for their exceptional ability to concentrate iodine from seawater. The ratio of iodine content between kelps (i.e. Laminariales) and seawater reaches 10^5, especially in young plantlets (Leblanc et al, 2006). Moreover, brown algae are also known to release volatile iodine compounds in response to various stresses, such as during emersion at low tide (Ball et al, 2010; McFiggans et al, 2010; McFiggans et al, 2004; O'Dowd et al, 2002; Whitehead et al, 2009). Wherever brown seaweed is exposed to ^{129}I discharges in seawater, the radioisotope could return to the terrestrial environment via an airborne route, namely via the wind and aerosols. For these reasons, brown seaweed is routinely collected in the marine environment in the English Channel to monitor radionuclide concentrations, and in particular that of ^{129}I (Fiévet et al, 2020). In a previous study on radionuclide transfers between seawater and biota, we observed that the bioavailability of ^{129}I appeared to decrease with distance from the source of input (the ORANO La Hague plant outlet). We emphasized the role of the chemical speciation of dissolved iodine in seawater (Fiévet et al, 2021). Iodine uptake by Laminariales, and more generally brown algae, is hypothesized to be mediated by enzymes from the vanadium-haloperoxidase (VHPO) family, through the specific oxidation of iodide (Küpper et al, 1998). Dissolved iodine is naturally present in seawater in the forms of iodide (I^-) and iodate (IO_3^-). Although iodate is the dominant chemical form because of pH and redox potential in seawater, both chemical forms are present in various proportions (Carpenter et al, 2013; Hou et al, 2007; Wong, 1991). The conversion mechanisms between iodine species are still unclear because iodate does not spontaneously reduce to iodide in natural seawater for thermodynamic reasons (Hou et al, 2007). Nevertheless this reaction between iodate and iodide takes time to reach equilibrium (Luther et al, 1995), around two weeks according to estimates (Carpenter, pers. Comm.). Now, depending on the chemical form of ^{129}I discharged by the reprocessing plant, the question arises as to its bioavailability for seaweed. The PUREX process (https://www.cea.fr/Documents/monographies/Procédé-PUREX.pdf) operated at the ORANO reprocessing plant results in the liquid release of ^{129}I as iodide. As such, it is assumed to be a potential substrate for VHPOs, being incorporated by kelps. But as conversion into $^{129}IO_3^-$ occurs in seawater, it may become unavailable for the enzyme. In summary, two crucial questions should be answered to better understand ^{129}I uptake by brown seaweed in the marine environment around the reprocessing plant of ORANO La Hague: 1- Which form of iodine is bioavailable for uptake by brown seaweed? 2- Does it evolve with time and distance from the outlet once released in seawater by the plant? More generally, the exact mechanism of iodine uptake is still unclear, and was challenged by chemical imaging results confirming a major apoplastic storage location (Lebeau et al, 2021). Assessing which chemical form of seawater iodine is taken up by kelps in the marine environment should contribute to the exploration of its bioaccumulation mechanism.

In this study, we investigate *in vivo* iodine uptake in *L. digitata* with an emphasis on chemical speciation between iodide *vs* iodate. We also use radioactive $^{131}I^-$ as a tracer of unidirectional iodide influx to estimate iodide turnover in seaweed as well as the relationship with seawater iodide concentration. We also report an isotopic ratio $^{129}I/^{127}I$ measured in the marine environment in the vicinity of the outlet of the ORANO reprocessing plant in La Hague. Iodine-129 activity and stable iodine concentrations were determined in total dissolved iodine in seawater and as total iodine extracted from two species of brown seaweed, *L. digitata* and *Fucus serratus* collected in Goury (6 Km from the plant outlet). Finally, laboratory experiment results and data from the natural environment are put into perspective to attempt to better understand radioactive iodine transfers between seawater and brown seaweed.

2 Material and methods

2.1 Algal material

For iodine uptake experiments, plantlets of *Laminaria digitata* [0.05-3 g] were collected on the rocks in Goury (Normandy, France) at low tide (spring-tides) and transferred to the laboratory (LRC, IRSN, Cherbourg, France) for maintenance in an aquarium. The temperature was set to that of the natural environment upon collection (12.5 °C), the water was aerated and recirculated. About 200 g of total biomass (plantlets and young sporophytes, see below) was kept in a 60 L tank in natural light, and the seawater was replaced every week with pre-cooled fresh seawater. The iodine concentration in seawater (see 2.2.2) was checked to ensure no depletion occurred.

For ^{131}I experiments, we used both whole plantlets and tissue disks. In the latter case, disks of about 0.04-0.05 g (diam. 14 mm) were punched out of the blade of young sporophytes (20-40 cm long; collected and maintained as described above) of *L. digitata* by die-cutting. Algal disks were then kept in filtered seawater (seawater collected in Goury, filtered at 0.45 µm; Millipore ref. HAWG0700) until iodine leakage stopped, as indicated by the monitoring of iodine concentration in seawater (with one disk in 10 mL of seawater: no iodine leakage was observed after 1 hour).

For the environmental monitoring of $^{129}I/^{127}I$, adult sporophytes of *L. digitata* (1-1.5 m in length) and *Fucus serratus* (30-70 cm in length) were collected monthly at low tide at Goury for 37 months (see supplementary material S2-1 for sampling location).

2.2 Determination of I^- and IO_3^- in seawater

2.2.1 Separation of seawater I^- and IO_3^-

After sample filtration (0.45 µm, Millipore ref. HAWG0700), seawater I^- and IO_3^- were separated by anion exchange chromatography (AEC) using Bio-Rad AG1-X4 resin and mini-spin columns according to (Hou et al, 2001; Zhang, 2015). Briefly, AG1-X4 resin (provided in Cl^- form) was conditioned into NO_3^- form as follows: 1- a first wash in NaClO 5% removed any residual I^- from the fresh resin. 2- Cl^- was replaced by NO_3^- in $NaNO_3$ 2M. 3- Excess NO_3^- was finally flushed with MilliQ water (Millipore). About 0.5 cc of conditioned resin (NO_3^- form) was added to mini Bio-spin columns (Bio-Rad #7326207) and allowed to drain by gravity. Then 3 more 0.9 mL washes of MilliQ water were applied and left to drain prior to starting AEC on filtered (0.45 µm) seawater as follows: 1- two 0.9 mL seawater samples were passed through the resin to replace the mobile phase. 2- The bottom of the column was closed with the stopper tip, another 0.9 mL sample was added and the top was closed with the cap. The column was flipped twice and agitated horizontally for 1 hour on a 3D-platform shaker (Polymax) at slow speed. 3- The bottom stopper tip and the top cap were removed and the mobile phase was allowed to drain in a 1.5 mL centrifuge tube. The flow-through contained IO_3^- anions whilst I^- anions stayed trapped on the resin. Total dissolved iodine concentration was measured (see 2.2.2) before the AEC (I^-

+ IO_3^-) and in the column flow-through (IO_3^-), I^- was calculated as the difference. We checked the accuracy of the separation with the standard addition method using KI, KIO_3 and both together.

2.2.2 Determination of iodine concentration

Total dissolved iodine (as I^- and/or IO_3^-) concentration in seawater was determined by spectrophotometry according to (Sandell & Kolthoff, 1937) in the range [0.04-0.30] µM. Seawater samples were filtered (0.45 µm, Millipore ref. HAWG0700) and diluted in MilliQ water to fit within that range. The color reaction was prepared in a 1.5 mL centrifuge tube with 500 µL of either seawater or an AEC-treated seawater sample (diluted), 50 µL NaCl (10% g/g), 500 µL As_2O_3 (0.1 M) and transferred to a water bath at 30°C. Then 125 µL of $(S0_4)_4Ce(NH_4)_4$, 2 H_20, (0.02 M) was added and the reaction was allowed to run for exactly 20 min at 30°C in the water bath. Absorbance at 420 nm was then measured in 1 mL disposable cuvettes in a spectrophotometer. A calibration curve was carried out with standard solutions between 0.039 µM and 0.316 µM (dilutions of 1.31 $g.L^{-1}$ KI in MilliQ water solution). The repeatability of the method showed that the uncertainty of the results was ± 5%. Total iodine concentration before (I^- + IO_3^-) and after (IO_3^-) separation by AEC (see 2.2.1) was determined in order to obtain the I^- concentration based on the difference. We checked the accuracy of the quantification with the standard addition method using KI, KIO_3 and both together. The reliability of method was further checked by comparing the results obtained by spectrophotometry with duplicates analyzed by ICPMS measurements on some of our samples.

2.3 Measurement of net iodine flux between seaweed and seawater

Plantlets of *L. digitata* (pooled to about 2 g) were incubated in beakers with 50-75 mL of filtered seawater (0.45 µm, Millipore ref. HAWG0700) at the same temperature as in the host aquarium (12.5 ± 1.0 °C), with bubbling. Seawater samples were collected over time (a picture of the incubation setup is provided in the supplementary material S1-1). The sample volume was 0.5 mL for total dissolved iodine (I^- + IO_3^-) and 3 mL for AEC separation. Beakers without seaweed plantlets served as controls for changes in iodine concentrations into the filtered seawater used due to adsorption onto the beakers surface. No changes in iodine concentrations were observed in those beakers. At some point in the experiment, seawater was spiked either with KI, KIO_3 or both. Upon completion of each experiment, the plantlets were patted dry between absorbent paper sheets and weighed. Concentrations of both I^- and IO_3^- in the water were plotted as a function of time.

2.4 Measurement of unidirectional $^{131}I^-$ influx in seaweed

Iodine-131 as KI was purchased from CIS Bio International (I-131-S-2) and the activity information was provided by the manufacturer with the calibration date (Act.=1.11E+08 Bq; Vol.=0.67 cc; $^{131}I/^{127}I$=4.64E+08). Because of constraints in accurate nuclear metrology (geometry calibration), unidirectional radioactive ^{131}I influx was measured on tissue disks punched out of *L. digitata* blade as previously published (Küpper et al, 1998; Shaw, 1959). Tissue disks were incubated in seawater spiked with ^{131}I in the form of KI. The disks were rapidly patted dry on absorbent paper, transferred to a plastic container and counted for 60 sec on a hyper pure GeHP gamma detector. The measurement procedure lasted 1.5 min and the disks were then returned to the incubation medium to carry on the uptake kinetics experiment. Radioactive iodine disk content was then plotted as a function of time and it was found to be linear for 15 min. The activity in seawater was found to be constant for short-term experiments (15 min). We investigated the relationship between the ^{131}I uptake by the disks and iodine concentration in seawater. For that purpose, seawater iodine concentration was increased by addition of KI to 1, 3, 10, 50, 500 µM and spiked with ^{131}I (230 $Bq.mL^{-1}$). The slope of the 15 min linear ^{131}I uptake allowed the iodine permeability of the disks to be estimated (in $nmol.min^{-1}.g^{-1}$) since the exchange

surface was constant. Plotting the iodine permeability as a function of seawater concentration allowed for the derivation of the values of Vmax and Km according to the Michaelis-Menten model as previously described by (Küpper et al, 1998). The Michaelis-Menten model was fitted using Microsoft Excel™ Solver function.

A longer time intake experiment was carried out over 14 days with a 0.1 g plantlet kept in a 10 L tank to estimate iodine turnover by the algae. The procedure was the same as for tissue disks with the plantlet patted dry and lying in a plastic dish for gamma counting. The activity of the seawater (30 $Bq.mL^{-1}$ seawater) was maintained constant by adding ^{131}I to compensate for intake by the plantlet as well as ^{131}I decay. The very large volume of the tank compared to the small size of the plantlet ensured that no total iodine depletion occurred in seawater. The biological half-life of iodine in *L. digitata* plantlet was estimated as $tb_{1/2} = Ln(2)/k$ by fitting the data to equation $Act_t = Act_0.[1-exp^{(-k.t)}]$ (with t=time; Act=^{131}I activity at time t; k=time constant) with the Solver function of Microsoft Excel™ (we don't need to implement ^{131}I decay since the radioisotope activity is artificially maintained constant in seawater).

2.5 Measurement of iodine-129 activity in seawater

120 L-seawater samples were collected monthly at Goury for 37 months, and filtered at 0.45 µm (Millipore, Durapore membrane ref. HVLP2932A). Total dissolved iodine concentration was determined in an aliquot as described above (2.2.2). A known amount of stable iodine (0.500 g KIO_3) was added to the 120 L as a carrier and to allow for the calculation of the extraction yield. Dissolved iodine in the forms of I^- and IO_3^- were merged by the addition of 120 g Na_2SO_3 at pH 2 (HCl-36%) to reduce IO_3^- into I^-. Total I^- was then precipitated by the addition of 6 g $AgNO_3$. The AgI precipitate was recovered in 500 mL with MilliQ water and then solubilized by the addition of 35 g L+ascorbic acid ($C_6H_8O_6$) at pH 10 (NaOH 5N). The solution containing dissolved iodine was then transferred into a separation funnel and covered with 100 mL 1,2,4-Trimethylbenzene (TMB). HNO_3 65% was added until pH 2 was reached, and $NaNO_2$ added whilst pH was maintained with HNO_3 to oxidize iodide into I_2. The funnel was vigorously shaken to trap I_2 in the organic phase and then left to decant. The aqueous phase was recovered and another 50 mL TMB was added to further extract I_2. The two TMB organic phases were pooled (150 mL) and washed twice with 100 mL MilliQ water (the water was discarded). Extracted iodine was finally recovered from the organic phase by adding 20 mL of NaOH 0.5 N; Na_2SO_3 0.2 M to reduce I_2 into I^-. The separation funnel was vigorously shaken, allowed to settle, and the 20 mL aqueous phase was recovered in a plastic vial where traces of TMB adhered to the plastic wall. The aqueous phase was finally transferred into a clean 20 cc geometry. The final 20 cc geometry contained initial + added stable iodine and unknown iodine-129. Iodine-129 activity was determined by gamma spectrometry in the 20 cc geometry previously calibrated with a standard solution (IL29-ELSF15 #4793/4, provided by CEA/DAMRI), using a hyper pure GeHP gamma detector (29,78 keV double ray, emission rate 57,3%, detection limit = 0.05 Bq in the final sample with a counting time of 80 000 s, 0.03 Bq in the final sample with a counting time of 240 000 s). A quenching curve was obtained by incrementing stable iodine concentration in the standard IL29-ELSF15 solution using the same 20 cc geometry: the quenching by stable iodine was lower than 15% (15% at 600 mg iodine, 5% at 200 mg iodine). Stable iodine concentration in the 20 cc geometry was determined by potentiometric method using an Orion 96-53 ionplus® Series Iodide Electrode according to the manufacturer's instructions. The iodine concentration in the geometry was used to correct the results for the extraction yield and for gamma counting quenching. Extraction yield was in the range [70%-80%]. Final results were expressed in $Bq.m^{-3}$ for ^{129}I and in µM for stable ^{127}I. The isotopic ratio $^{129}I/^{127}I$ could then be calculated in seawater for total dissolved iodine ($I^- + IO_3^-$; with 1 Bq ^{129}I = 1.186E-09 mol).

2.6 Iodine-129 activity measurement in seaweed

In parallel with seawater, iodine-129 activity was determined in *L. digitata* and *F. serratus* samples collected monthly (see **Erreur ! Source du renvoi introuvable.**), dried at 90°C and finely ground as described in (Bouisset et al, 1999; Lefevre et al, 2003). In both species, stable total iodine concentration was determined in parallel on aliquots by the CNRS/SCA (Service Central d'Analyses, Vernaison, France). Seaweed dry material was mineralized in Schöninger combustion flasks and the solutions were analyzed using ion exchange chromatography with electrochemical detection (the quantification limit was 0.25 µg.L^{-1} iodine). Final results were expressed in Bq.Kg^{-1} dry weight for ^{129}I and in mg.Kg^{-1} dry for stable ^{127}I. The isotopic ratio $^{129}I/^{127}I$ could then be calculated in dry seaweed material).

3 Results

3.1 Which form of inorganic iodine in seawater is taken up by *Laminaria digitata*?

A first series of experiments consisted in the incubation of *L. digitata* plantlets in natural seawater collected in Goury at the same location as the seaweed with [IO_3^-] = 0.35 µM and [I^-] = 0.25 µM. The changes in seawater IO_3^- and I^- concentrations showed that only IO_3^- appeared to decrease in the medium in the presence of the plantlet, and this was confirmed by spiking seawater with IO_3^- at time 90 min (Figure 1 left). However, spiking seawater with I^- instead of IO_3^- clearly demonstrated that both iodine forms decreased (Figure 1 right). These experiments were replicated 6 times for I^- and IO_3^- independently with the same results (supplementary materials S1-2 and S1-3).

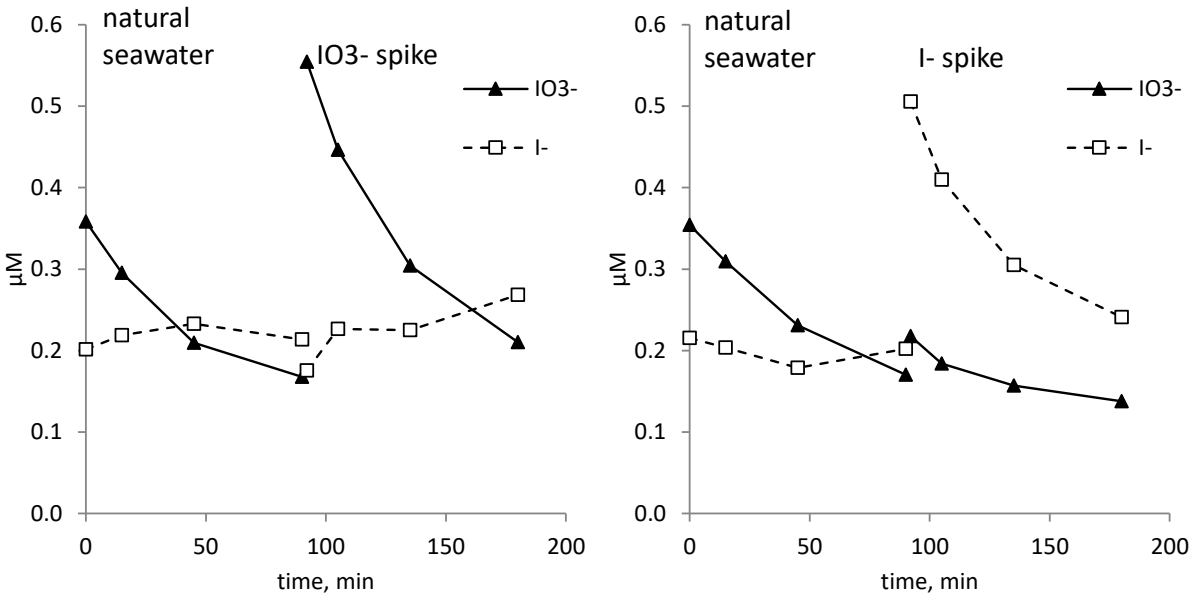

Figure 1: Incubation of *Laminaria digitata* plantlets: changes in seawater IO_3^- (filled triangle) and I^- (open square) concentrations over time. The uncertainty of the measurements was ± 5 % (error bars omitted to ensure clarity). Seawater was spiked with IO_3^- (left) and I^- (right) after 90 min of incubation.

When replicating the experiment, the transfer of *L. digitata* plantlet to the incubation vial sometimes resulted in transient iodine leaks from the seaweed. In those cases, only I^- increased in seawater whilst IO_3^- continued to decline (Figure 2).

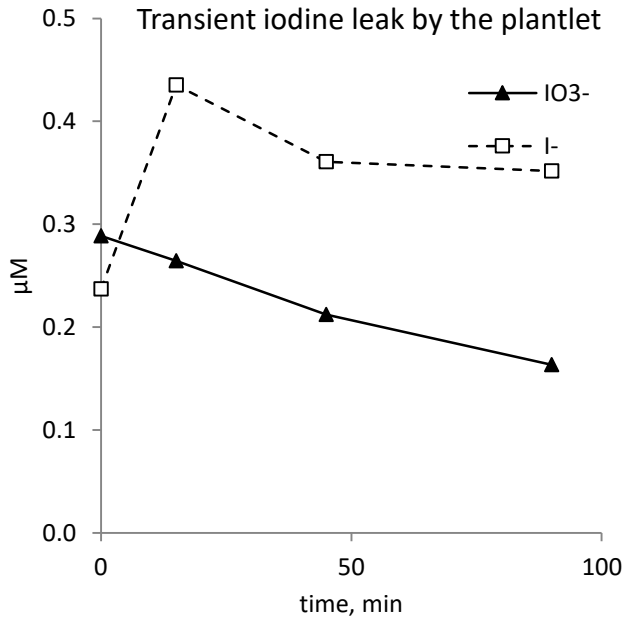

Figure 2: Transient iodine leak by the plantlet which only resulted in net I^- output whilst IO_3^- was still taken up (same symbols as in Figure 1, uncertainties ± 5 %).

Since both IO_3^- and I^- seem to be taken up by *L. digitata*, the question then arose as to why the I^- concentration didn't decrease in the experiment illustrated in Fig.1 left? A simple hypothesis is that the concentrations in seawater determine which form of iodine is pumped. We repeated the experiment with different concentrations of IO_3^- and I^- in seawater, starting with the same concentrations of both forms of iodine, and increased the levels up to 1.4 µM for each iodine species (Figure 3).

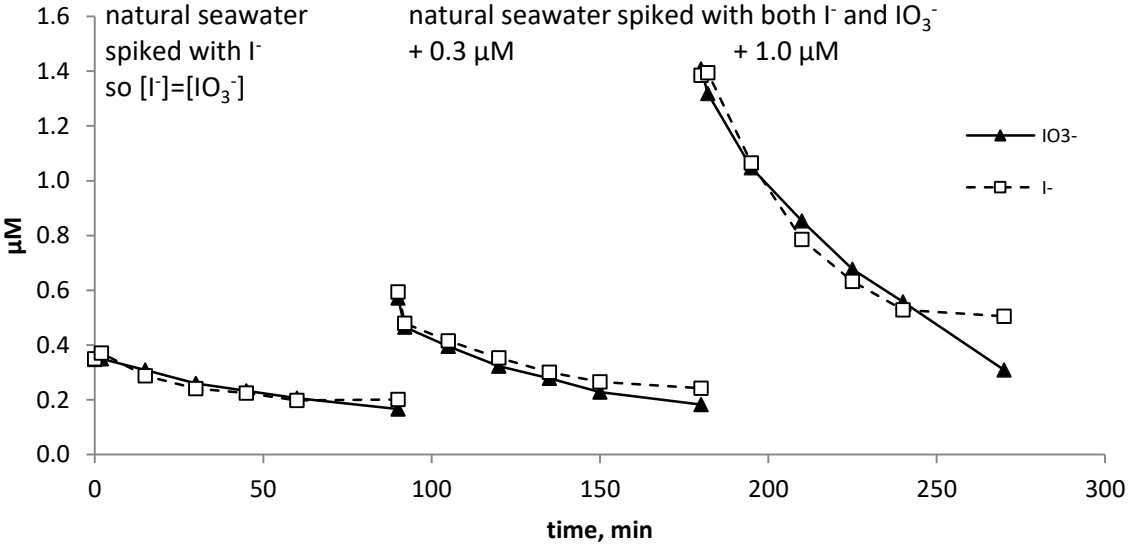

Figure 3: Incubating *Laminaria digitata* plantlets in seawater with increasing levels of IO_3^- and I^- at the same concentration (same symbols as in Figure 1, uncertainties ± 5 %).

When both IO_3^- and I^- were present at the same concentration, their concentrations decreased equally in the presence of the plantlet. So, in the left experiment in Figure 1, was there a minimum I^- concentration below which the uptake just balanced the output? Likewise, does a minimum IO_3^- concentration exist below which this iodine form uptake just balances the output? To address this

point, we extended the net flux experiment for 6 hours to attempt to see the "asymptotic" seawater IO_3^- and I^- concentrations when no more net flux were observed. At the end, the net iodine uptake was re-stimulated by a fresh spike of I^- and IO_3^-. The results of this long-term kinetics are shown in Figure 4.

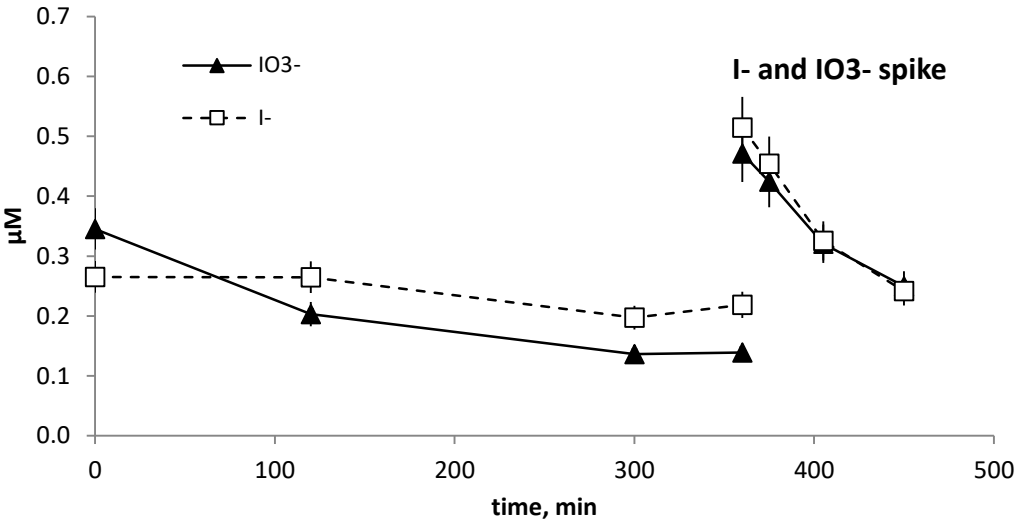

Figure 4: Long-term kinetics experiment on net iodine flux using plantlets of *Laminaria digitata* (same symbols as in Figure 1).

This long-term kinetics experiment was replicated 7 times (supplementary materials S1-4) and every time, IO_3^- concentrations declined below I concentrations$^-$, as in the example displayed in Figure 4. The average iodine concentrations (± SE) at time 0 were $[IO_3^-]$ = 0.35 ± 0.01 µM; $[I^-]$ = 0.31 ± 0.06 µM and at time 360 min, they were $[IO_3^-]$ = 0.16 ± 0.03 µM; $[I^-]$ = 0.29 ± 0.03 µM, (N=7).

3.2 Tracing iodine uptake in *Laminaria digitata* with ^{131}I

Since both IO_3^- and I^- seemed to be taken up equally by the seaweed (provided their concentrations were high enough), using radioactive $^{131}I^-$ was considered to trace dissolved iodine uptake whatever the chemical form. We investigated the relationship between the uptake and iodine concentration for *L. digitata* using disks punched out from the thallus blade as previously described (Küpper et al, 1998; Shaw, 1959). The unidirectional flux J_{in} (nmol.min^{-1}.g^{-1}) was proportional to seaweed iodine permeability, since the disk surface was constant during the experiment. The results are presented in Figure 5 fitted with Michaelis-Menten model kinetics with apparent Km = 59 µM and Vmax = 216 nmol.min^{-1}.g^{-1}. From those results, we calculate an approximate unidirectional influx capacity of ^{131}I of c.a. 100 nmol.min^{-1}.g^{-1}. This unidirectional influx can be compared to results obtained in our net fluxes experiments (Figures 1 to 4), in which we obtained influxes of around 1 nmol.min^{-1}.g^{-1}. Net fluxes result from the balance between input and output fluxes. Therefore, the 2 orders of magnitude higher unidirectional input fluxes measured in our ^{131}I uptake experiment indicate that output fluxes must be very high to result in a 100 fold reduction when considering net fluxes.

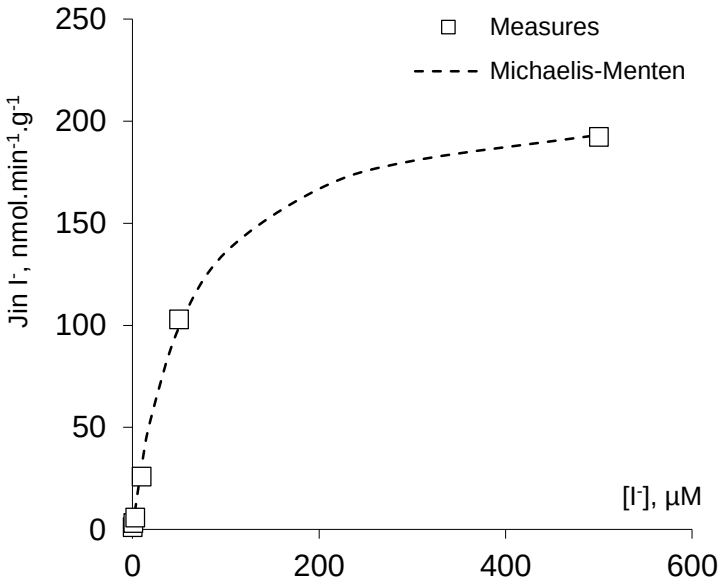

Figure 5: Relationship between unidirectional iodine influx and seawater iodine concentration for tissue disks of *Laminaria digitata*. Fitting the Michaelis-Menten model kinetics yielded Km = 59 µM and Vmax = 216 $nmol.min^{-1}.g^{-1}$.

A two-week experiment was performed to estimate the biological half-life of iodine in *L. digitata* plantlets. The ^{131}I uptake experiment displayed in Figure 6 yielded an estimated $tb_{1/2}$ value of c.a. 5 days.

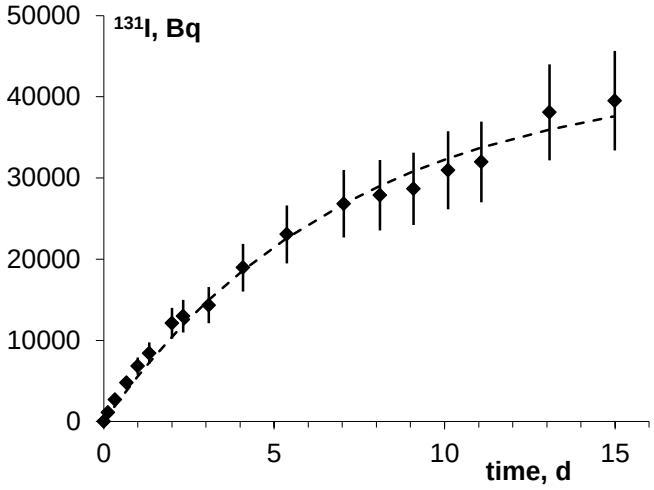

Figure 6: ^{131}I uptake over two weeks by *Laminaria digitata* plantlet leading to the estimation of the iodine turnover in the seaweed as 5 days.

3.3 ^{129}I transfer between seawater and brown seaweed exposed to discharges from the ORANO reprocessing plant in La Hague

3.3.1 Seasonal changes of iodine in seawater and in brown seaweed

Seawater samples were periodically collected in Goury and analyzed for iodine chemical speciation. Additional samples were collected in different locations around the Cap of La Hague and along the coast of Brittany (Bretagne, France). The data are presented in the supplementary material (S2-2).

Since brown seaweed iodine content may vary during the seasonal cycle, it was crucial to take these changes into account when looking at radioactive iodine transfer to seaweed. Total iodine in $mg.Kg^{-1}$ dry was determined in *L. digitata* and *F. serratus* samples were collected monthly in Goury. The data are displayed in the supplementary material (S2-3).

3.3.2 Iodine-129 activity in seawater and in brown seaweed

Iodine-129 activity determined in dissolved iodine in seawater (IO_3^- + I^-) and in the two brown seaweed species *L. digitata* and *F. serratus* are presented in Figure 7.

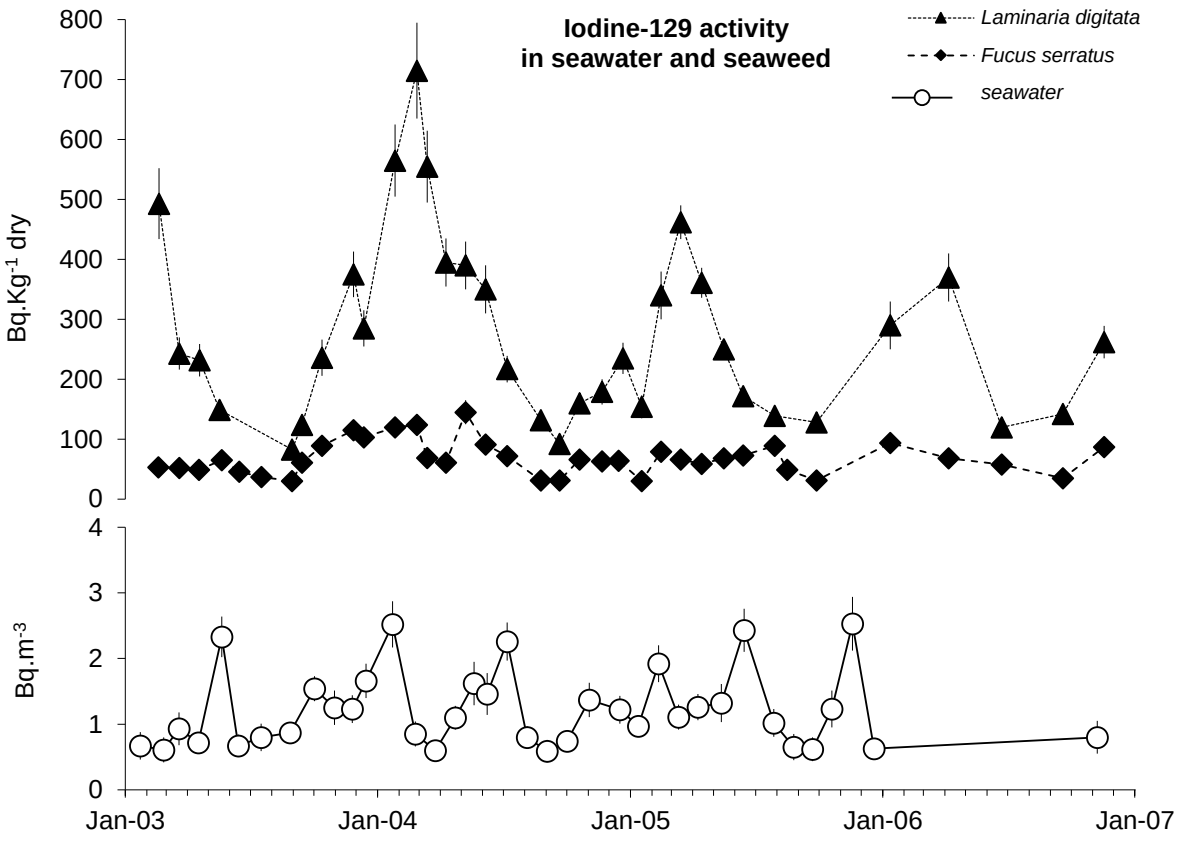

Figure 7: Iodine-129 activity in seawater and in brown seaweed collected in Goury, near the outlet of the ORANO reprocessing plant in La Hague. Error bars account for metrological uncertainties (2 x sigma; may be masked by symbol).

Because iodine content varies in seaweed over the season, expressing the radioactivity data as the isotopic ratio $^{129}I/^{127}I$ was necessary to explore ^{129}I transfer between seawater and seaweed. The ratio $^{129}I/^{127}I$ was determined in dissolved iodine in seawater (I^- + IO_3^-) and in *L. digitata* and *F. serratus* dry material and the data are presented in Figure 8.

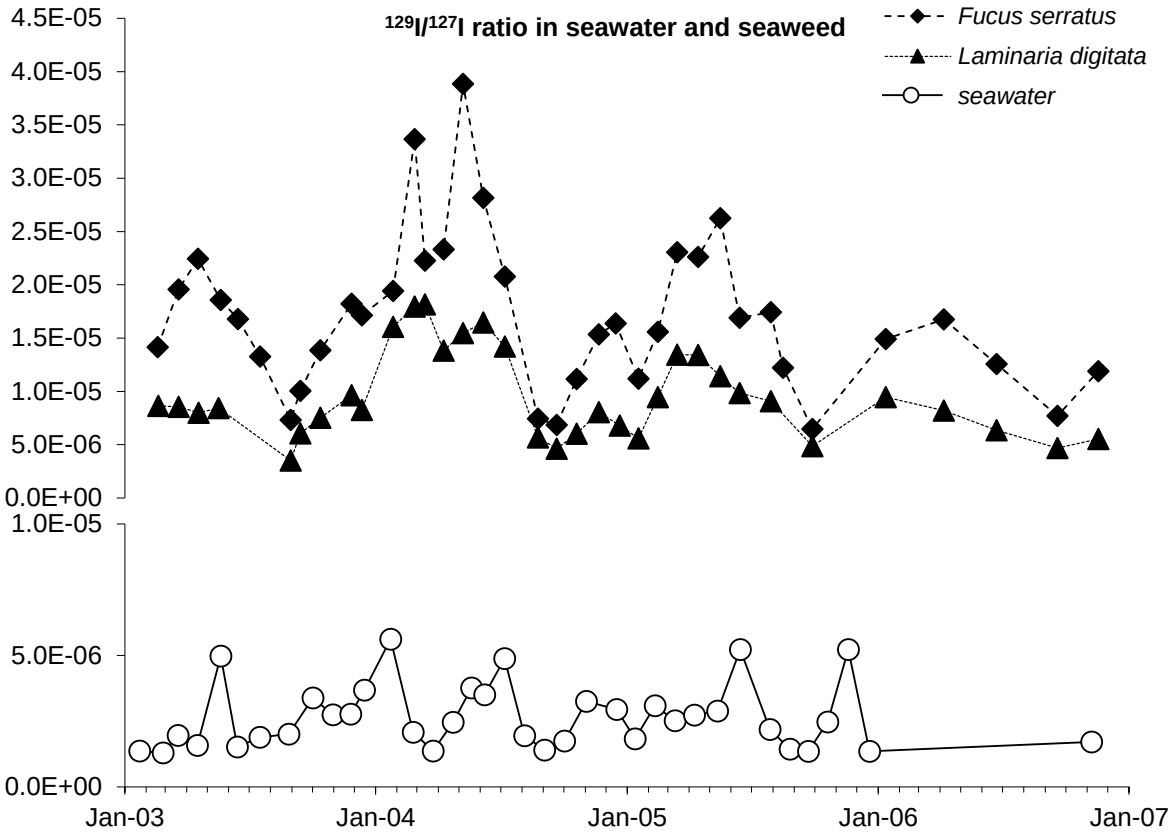

Figure 8: Iodine-129 isotopic ratio in seawater and in brown seaweed collected in Goury, near the outlet of the ORANO reprocessing plant in La Hague.

4 Discussion

The fate of ^{129}I liquid discharged by the ORANO reprocessing plant in La Hague (Normandy, France) deserves special attention for several reasons: due to its very slow decay, this radionuclide is a major residual iodine radioisotope in the nuclear fuel cycle and a tracer of the discharges by the ORANO reprocessing plant in the environment. As part of the liquid waste released in seawater in the Alderney Race (Bailly du Bois et al, 2020; Fiévet et al, 2020), it mixes with natural stable iodine so that isotopic dilution minimizes its impact in terms of radioprotection. However, brown seaweed is known to be exceptionally efficient at bioaccumulating iodine from seawater. The recommended value for the iodine concentration factor (CF) in macroalgae is 10^4 (IAEA, 2004), and ratios of up to 4.3 10^4 were observed in kelps (Fiévet et al, 2021). Moreover, brown seaweed has the ability to transform accumulated iodine into volatile compounds, such as I_2, released in response to various stresses, such as emersion at low tide (Ball et al, 2010; McFiggans et al, 2010; McFiggans et al, 2004; O'Dowd et al, 2002; Whitehead et al, 2009). This process might challenge the strategy to keep ^{129}I away from humans because the isotope can return to the terrestrial environment *via* airborne routes *via* the wind and aerosols. For these reasons, although the potential contribution of this atmospheric route is not known, brown seaweed is focused on as part of radionuclide monitoring of the marine environment. Understanding the transfer of radioactive iodine released by the nuclear industry (under control) in the marine environment is not only essential for the sake of radioprotection, the fate of radioactive iodine released into the English Channel also has a much larger scope than radioprotection as a potential tracer of the complex behavior of iodine in the marine environment. The different key steps of iodine transfer from seawater to the bioaccumulation in brown seaweed are part of the bigger puzzle of the biogeochemical cycle of iodine in the ocean and its consequences on the chemistry of the atmosphere (Carpenter et al, 2021). The transfer of iodine from the ocean to the atmosphere is

assumed to occur through the oxidation of iodide (I^-) into diiodine (I_2) in the presence of atmospheric ozone under solar radiation, but also through biological oxidation processes (Carpenter et al, 2021). Iodate (IO_3^-) is the preferential chemical form of iodine in seawater, therefore its conversion into iodide (I^-) is not spontaneous as regards thermodynamics (Hou et al, 2007; Wong, 1991). After decades of literature on the topic, the reduction of iodate into iodide still remains poorly understood, though microorganisms or marine algae clearly play a role (Carpenter et al, 2021; Wong, 1991) and this factor has recently been confirmed for some marine bacteria (Reyes-Umana et al, 2022).

In the present study, we provide new information on the uptake mechanism of iodine by kelp (*L. digitata*). First, our results suggest that kelp plantlets were able to take up iodine in the forms of both IO_3^- and I^- with the same efficiency (Figure 1). When both iodine species were present at the same concentration, they declined at equivalent rates in seawater (Figure 3). It should be emphasized that our experimental conditions magnified naturally occurring phenomena due to a change in the ratio between the seaweed mass and the volume of its surrounding seawater compared to the natural environment. This magnification allowed us to easily assess the net IO_3^- and I^- fluxes between the plantlet and seawater by measuring the decrease in those iodine species in seawater, with our measurements accounting for the balance between inputs and outputs. Plantlets of *L. digitata* are very sensitive and required extreme care during transfers from the natural environment and between the host aquarium and the flux experiment setup. Chemical imaging data provided information on a very unequal compartmentalization of iodine in the tissue of *L. digitata* plantlet. Iodine appears to be preferentially concentrated in the peripheral apoplastic tissue (Lebeau et al, 2021). As such, transient iodine leakage might occur when manipulating a plantlet. We assumed that plantlets underwent minimal stress because after each experiment, they were returned to the host aquarium and could be re-used for more experiments, and the iodine leaks sometimes observed at the beginning of the experiments were only transient and brief. Indeed, a transient iodine leak by the plantlets was observed in 11 out of 42 net flux experiments. In all cases it should be emphasized that only I^- leaks were ever detected in seawater whilst IO_3^- always continued to decline (Figure 2). This observation was an *in vivo* evidence that iodine is stored in *L. digitata* tissue as iodide (I^-), as shown by laser desorption/ionization mass spectrometry (Lebeau et al, 2021). We could then interpret the results of our net flux experiment on the basis of this information. When assessing IO_3^- and I^- net fluxes, we expected those fluxes to stop when the inputs balanced the outputs, or when the seawater concentrations were too low to meet the needs of the uptake process. In the long-term (6 hours) experiment (Figure 4), a change in seawater iodine speciation was observed in the presence of *L. digitata* plantlets. The IO_3^- concentration was initially above the I^- concentration, and this reversed after 2 hours. IO_3^- declined down to around 0.15 µM whilst I^- stayed at around 0.25 µM. If the seaweed only stores I^- but can take up both IO_3^- and I^-, we conclude that IO_3^- was likely to be converted into I^- at some point in the plantlet (apoplast), or through the action of associated microorganisms. After 5-6 hours of incubation, the stable IO_3^- and I^- concentrations in seawater therefore resulted from IO_3^- + I^- influxes and I^- outflux only. We assume that the IO_3^- stabilized in seawater because its concentration was so low that the uptake mechanism was no longer able to pump this iodine form, "by exhaustion", as indicated by the more than 200 fold difference between the K_M of 59 µM and the final iodine concentrations of 0.15-0.25 µM we measured. Conversely, the extreme chemical gradient of I^- between the seaweed and seawater and its permeability resulted in I^- outflux. I^- stabilization in seawater corresponded to the balance between this I^- leak and the ability of the mechanism to take up I^-. So, when the IO_3^- is low enough in seawater to exhaust its uptake, we only observe I^- recycling. A major conclusion of this laboratory experiment was that we directly observed the kelp-based biogenic conversion of seawater IO_3^- into I^-. Even if the quantitative contribution of the process was considerably

magnified with our biomass/seawater ratio, this provided direct evidence of this iodine conversion mediated by brown algae and associated microorganisms observed in the marine environment.

Second, we also concluded that *L. digitata* plantlets can take up either IO_3^- or I^- indifferently. Iodine bioaccumulation in kelps is assumed to be mediated by vanadium-dependent haloperoxidases (VHPO) family (Küpper et al, 1998), and especially by vanadium-dependent iodoperoxidases (VIPO) characterized in *L. digitata*, specific to iodide oxidation (Colin et al, 2005). The substrates of vHPOs are halides and H_2O_2 (Colpas et al, 1996) so VIPO is assumed to oxidize I^- and not IO_3^-. The hypothetical mechanism involving only VIPO-based iodide uptake in iodine bioaccumulation by kelp was recently questioned by our data on the apoplastic localization of iodine storage in the plantlets (Lebeau et al, 2021; Verhaeghe et al, 2008). The parallel uptake of IO_3^- and I^- that we observed in our net flux experiments suggests a need to further validate *in vivo* the biological function of VHPO and/or VIPO in iodine bioaccumulation. However, we suggest the following speculative scheme: if iodide is the only form of iodine in the storage site, as shown previously (Lebeau et al, 2021; Verhaeghe et al, 2008), oxidized forms may have a favorable chemical gradient to enter the seaweed. This would rely on the strong reduction capacity of the storage site in order to maintain this gradient. The question of this reduction capacity inside the seaweed storage compartment is still pending, but in this scheme, the driving force would be this reduction capacity of the apoplastic storage site and the uptake would only rely on oxidized iodine accessing the site along a favorable chemical gradient. Natural IO_3^- in seawater would be available for this, and VIPO would be there to oxidize I^- when present. It should be kept in mind that a major role for the I^- bioaccumulated in the seaweed is to react with reactive oxygen species to reduce oxidative stress and/or to release oxidized iodine in response to stress as a defense mechanism (Küpper et al, 2008).

We used an ^{131}I tracer to further characterize iodine uptake in kelp. This replicated a kinetic experiment (3 hours) performed many decades ago (Shaw, 1959). The isotope was added in the form of $^{131}I^-$, but our net flux experiment showed that both IO_3^- and I^- uptakes exactly matched. This suggested that $^{131}I^-$ unidirectional influx data were relevant to trace iodine uptake. The dependence of I^- uptake on concentration followed Michaelis-Menten kinetics, as previously published with disks of *L. digitata* (Küpper et al, 1998). The Michaelis-Menten kinetics confirmed the enzymatic mediation of I^- uptake but our values of Km and Vm parameters were quite different. Our Km value of 59 µM was more than 7 times lower and our Vm value of 219 µM was more than three time higher. We have no explanation for this discrepancy except maybe the physiological condition of the disks punched out from the blade of *L. digitata*. On the basis of our Km value of 59 µM, we may speculate that at concentrations more than two orders of magnitude lower, like in our flux experiments (Figure 4), the uptake mechanism reached a steady state. A biological half-life of 5 days for iodine was estimated based on the long-term $^{131}I^-$ uptake experiment on *L. digitata* plantlets. Although the values are consistent, this was below our previous estimate of 14 days in brown seaweed based on modeling using ^{129}I environmental data (*L. digitata* and *F. serratus*) in the marine environment in the vicinity of the ORANO reprocessing plant in La Hague (Fiévet et al, 2021). Our laboratory experiment lasted 15 days, but we can't rule out that we had not reached the plateau for ^{131}I activity in the seaweed. Besides, it was not surprising that the young plantlet of *L. digitata* showed small differences in iodine uptake kinetics compared to adult and other brown seaweed used in our environmental modelling.

The apparent convergence of IO_3^- and I^- uptake by *L. digitata* plantlets observed in our net flux experiments was essential for interpreting our study of radioactive iodine transfer between seawater and seaweed in the marine environment under the influence of radioactive liquid discharges from the ORANO reprocessing plant in La Hague. ^{129}I should be measured in seawater based on total iodine (IO_3^- + I^-) to account for ^{129}I bioavailability at least for kelps. Seasonal changes in iodine concentrations in

seawater and in seaweed were also essential to take into account. In seawater, if we merge all total (IO_3^- + I^-) dissolved iodine measurements we took in seawater along the coast of the English Channel and Brittany (Map in the supplementary material S2-1), the average concentration (± SE) was 0.54 ± 0.05 µM (N=56). Our recent measurements around the Cape of La Hague (supplementary material S2-2) with IO_3^- and I^- partitioning showed relatively stable IO_3^- concentration whilst I^- seemed to fluctuate over the year. Longer time-series measurements are required to confirm these chemical speciation data, but a question already arose from these I^- data. In seawater samples collected from the shore, where brown seaweed including kelp was present, our IO_3^- concentration measurements of around 0.3 µM were consistent with offshore values reported in the literature (Hou et al, 2007). But our I^- concentrations onshore were around 0.2 µM, whilst they were in the range [0.04-0.08] µM in the middle of the English Channel. Interestingly, iodide concentrations around 0.15 µM were reported at coastal sampling locations from North Germany (Hou et al, 2007). This difference in iodide partition between coastal and offshore waters is in agreement with previous reviews by (Wong, 1991; Wong, 2001; Wong & Cheng, 2001a; Wong & Cheng, 2001b). Did the presence of local brown algae fields influence the iodine chemical partition (IO_3^- *vs* I^-) in our coastal seawater sample? This question is now under further investigation (Carrano et al, 2021). Nevertheless, total dissolved iodine concentration data in seawater were used to calculate the isotopic ratio of ^{129}I in total iodine (IO_3^- + I^-) measurements in Goury (Figure 7 and Figure 8). Iodine content in brown seaweed clearly cycled with season, with higher levels in winter than in summer (supplementary material S2-3). Does a higher growth rate in summer overwhelm the iodine uptake capacity of the seaweed? Does solar exposure at low tide or other stresses (thallus grazing by marine organisms) result in more volatile iodine release and loss? Regardless of the cause, it was critical to consider iodine seasonal fluctuations in seaweed (Ar Gall et al, 2004) when investigating radioactive iodine transfer between seawater and seaweed. The isotopic ratio $^{129}I/^{127}I$ in seaweeds and in seawater gave us a better understanding of iodine transfer. Up to 10-fold higher isotopic ratios were found in seaweed *vs* seawater (Figure 8). Although the ^{129}I activities were lower in *F. serratus* than in *L. digitata* (Figure 7), the $^{129}I/^{127}I$ ratio values in the former were above those in the latter (Figure 8). The discrepancy between the isotopic ratio $^{129}I/^{127}I$ in seawater and brown seaweed was puzzling. Our net laboratory IO_3^- and I^- flux experiments in *L. digitata* plantlets showed that both forms of iodine are equally taken up by the seaweed. Therefore, a conversion of $^{129}I^-$ discharged by the reprocessing plant into $^{129}IO_3^-$ would not result in a change in $^{129}I^-$ bioavailability for kelp, as we had however proposed in our recent review (Fiévet et al, 2021). This also invalidates this hypothesis to explain why the apparent $^{129}I^-$ bioavailability declined with distance from the plant outlet, as we noticed in seaweed with regards to hydrodynamic dispersion modelling (Fiévet et al, 2021). We cannot currently explain the difference in the isotopic ratio $^{129}I/^{127}I$ between seawater and brown seaweed. Although we cannot rule out isotopic fractionation in the uptake mechanism, it seems unlikely with such a small relative difference in the nucleus mass of the two iodine isotopes. A likely explanation was that, when looking at IO_3^- + I^- in seawater, we missed an essential ^{129}I compartment (chemical form) in the marine environment to which brown seaweed are exposed, an iodine compartment that remains to be identified. The difference in the isotopic ratio $^{129}I/^{127}I$ between the two seaweed species that we analyzed may provide a clue. *F. serratus* grows in the mid intertidal zone whilst *L. digitata* grows at the very bottom. It means that the former emerges twice a day at low tide and the latter only a few times a month during spring tides. More frequent contact with the seawater surface and longer contact with the atmosphere occurs for *Fucus* than *Laminaria*. To test this hypothesis in a preliminary experimental setup, we detached adult *L. digitata* from the rocks and artificially maintained the seaweed in a floating net for 1 month (photo in the supplementary material S2-4). At the same location, we compared ^{129}I activities in natural specimens from the bottom with those from the net and observed a 50% increase (not shown). The thallus tissue was not visibly degraded, and an identical ^{40}K level was taken as an indication of regular mineral balance. This

experiment was anecdotal and should be confirmed and further characterized. The question raised by the different isotopic ratio $^{129}I/^{127}I$ between seawater and brown seaweed is still open, but the sea surface and its interface with the atmosphere may be a focal point when investigating iodine transfer to seaweed in the marine environment.

5 Conclusion

The present paper aimed to improve our understanding of iodine transfers between seawater and brown seaweed in the context of radioactive liquid discharges from nuclear facilities in the marine environment. We are left with a series of questions: 1- The iodine uptake mechanism in brown seaweed, which yields an exceptional bioaccumulation level, unknown in any other living organism, is still mysterious. The difference of five orders of magnitude in concentration with respect to seawater is even a low estimate, since iodine storage compartments are probably only peripheral in the apoplast, as suggested by previous results in *L. digitata* (Lebeau et al, 2021; Verhaeghe et al, 2008). However, our net IO_3^- and I^- flux experiments demonstrated that both iodine chemical forms are taken up by *L. digitata* plantlets. The role of iodoperoxidases identified in this species and hypothesized to be key to specific iodide uptake (Colin et al, 2005) remains to be further validated *in vivo*. In this new context, a reduction process is likely to be a key step for completing the picture. 2- No step in the radioactive iodine biological cycle between seawater and brown seaweed is expected to change the isotopic ratio, except unlikely isotopic fractionation. However, our environmental data on the isotopic ratio $^{129}I/^{127}I$ in seawater and brown seaweed exposed to liquid discharges from the ORANO reprocessing plant in La Hague showed an unexpected discrepancy. This means that this in situ monitoring certainly missed a key compartment involved in ^{129}I transfer between seawater and brown seaweed. The question raised in our previous review on radioactive transfers in the marine environment around the reprocessing plant (Fiévet et al, 2021) regarding the non-conservative hydrodynamic dispersion of ^{129}I also remains open. Although important questions remain to be addressed, our laboratory experiments provided a direct observation of the ability of marine brown algae to modify iodine chemical speciation in seawater and to convert seawater IO_3^- into I^-. The endogenous extracellular pathways through which seaweed modify iodine chemistry in seawater remain to be further investigated. This conversion is a key step in the Earth's biogeochemical iodine cycle between the Ocean and the Atmosphere (Carpenter et al, 2021).

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8 Supplementary material

Additional data are provided in two files: Supplementary_Material_1.pdf and Supplementary_Material_2.pdf.

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