High proportions of inorganic arsenic in *Laminaria digitata* but not in *Ascophyllum nodosum* samples from Ireland.

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Abstract

Seaweed can accumulate inorganic arsenic (iAs) from seawater as hydrogen arsenate ($HAsO_4^{2-}$) in place of the phosphate anion (HPO_4^{2-}). While it is rapidly metabolised to organoarsenic species, predominantly arsenosugars and arsenolipids, iAs may be present in seaweed biomass; this poses a potential health concern for consumers of seaweed products. Here, the distribution of total (As_{TOT}) and iAs was determined in different thallus parts of the kelp *Laminaria digitata* and the intertidal fucoid *Ascophyllum nodosum* (both Phaeophyceae) using inductively-coupled plasma mass spectrometry (ICP-MS) and high performance liquid chromatography –ICP-MS (HPLC-ICP-MS). As_{TOT} ranged from 36 to 131 mg kg^{-1} dry weight (DW) in *L. digitata,* and from 38 to 111 mg kg^{-1} DW in *A. nodosum,* with no statistically significant differences between different thallus parts. iAs was detected in all *A. nodosum* samples, comprising less than 1% of the As_{TOT} content. Concentrations of iAs in *L. digitata* were significantly higher, ranging from 2.2 to 87 mg kg^{-1}, increasing through the thallus from the stipe to the decaying distal blades. iAs comprised more than 50 % of As_{TOT} in the middle to decaying distal blades. This finding has potential implications for harvesting, processing and use of *Laminaria digitata* in agri-, food and health applications.

Keywords: Arsenic; Macroalgae; Speciation; HPLC-ICPMS; Brown seaweed.

1. Introduction

Arsenic (As) is a naturally occurring element that raises concern from both an environmental and a human health perspective. Globally, large quantities of arsenic compounds are released into the environment from natural sources and anthropogenic sources (Chilvers, and Peterson, 1987). Over 100 arsenic species have been identified in the marine environment (50 water-soluble compounds (Francesconi 2010) and 69 arsenolipids (Petursdottir et al. 2017)).. Arsenic is present in seawater predominantly in the inorganic forms arsenate (AsV) and arsenite (AsIII) (Saxe et al., 1964) and is taken up by marine organisms and converted to organic forms such as arsenosugars and arsenolipids. Inorganic arsenic (iAs) is much more toxic than the majority of organic forms, and many organoarsenicals, such as arsenobetaine, are considered to exhibit little or no toxicity to humans (Hughes et al., 2011). Long-term ingestion of low levels of iAs may cause a variety of health effects, including skin lesions, cancer, developmental toxicity, neurotoxicity, cardiovascular diseases, abnormal glucose metabolism and diabetes (EFSA, 2009, Hughes et al., 2011). The International Agency for Research on Cancer (IARC) classified arsenic and inorganic arsenic as Class 1, 'carcinogenic to humans'. (IARC, 2012). Recently, arsenolipids such as arsenic containing hydrocarbons have been shown to be as cytotoxic as iAs (Meyer et al., 2015) while other commonly occurring arsenolipids (arsenic containing phospholipids) in seaweeds have not been tested for their toxicological potential.

Seafood is a primary dietary source of arsenic contributing over 50 % of total daily dietary intake of arsenic (EFSA, 2009). This arsenic occurs, with some exceptions, in relatively non-toxic organic forms, and the actual risk to consumers cannot be assessed without data on the occurrence of individual As species or, at a minimum, data on the concentrations of the toxic inorganic forms (Amlund and Sloth, 2011). Algae contain the greatest number of arsenic species among marine samples. Arsenate can be taken up by algae in place of the phosphate anion, due to their similar pKa values. It is then rapidly transformed by a process of methylation and alkylation to form a number of end products, most commonly arsenosugars (Francesconi and Edmonds, 1998, Edmonds et al., 1997, Feldmann and Krupp, 2011). Algae also contain AsV, generally as a minor constituent, besides

arsenolipids and DMA in traces, with the exception of certain known algal species such as *Hijiki fusiforme*, where AsV may account for the majority of the arsenic present (Francesconi and Edmonds, 1998, Rose et al. 2007).

According to the FAO, the global seaweed industry is estimated to be worth US $6.4 billion annually (FAO, 2014) with seaweed and seaweed-derived products being used for human and animal consumption, hydrocolloids, plant supplements, specialist fertilisers, cosmetics and agricultural products (Stengel and Connan, 2015). The potential risk to consumers from seaweed derived iAs is difficult to determine. Seaweed presents a challenge to monitoring arsenic in food and assessing exposure due to its variable and complex As species distribution, and due to the difficulty in reliably quantifying As species in marine algae (Taylor and Jackson, 2016).

Internationally there are few regulatory limits for arsenic in seaweeds destined for human consumption or use in feed. Australia and New Zealand have established maximum levels of iAs of 1 mg kg^{-1} for seaweed and molluscs (Australia New Zealand Food Standards Authority, 2013). In Europe, Directive 2003/100/EC sets a maximum limit of 40 mg kg^{-1} (12 % moisture) for total arsenic (the sum of all As species present, As_{TOT}) in animal feed including seaweed meal and feed materials derived from seaweed. Currently there are no EC regulatory limits set for arsenic (both total and inorganic) in fish and seafood for human consumption although new limits for iAs are anticipated (Petursdottir et al., 2015). France have set a limit of 3 mg kg^{-1} iAs in seaweeds for food consumption (AFSSA, 2009).

The brown seaweeds (Phaeophyceae) *Laminaria digitata* (Hudson) J.V.Lamouroux (Laminariales) and *Ascophyllum nodosum* (L.) Le Jolis (Fucales) are commonly harvested for use in animal feed, fertiliser, alginate production, and, for *L. digitata*, as a sea vegetable (FAO, 1987) and directly used as feed for sheep or deer (Hansen et al., 2003). Some kelps are referred to ‘kombu’ when sold for human consumption, and are typically sold in dried form. Intra-thallus variation in chemical and biochemical composition of both brown algae has previously been demonstrated (e.g. Stengel et al 2005; Schmid

and Stengel, 2015) and was related to morphological differentiation into different structural thallus regions (e.g. holdfast, stipe, meristematic growth region at the base of the blade, young, mature and older distal blade sections for *L. digitata*; versus apically growing thalli with annual airbladders along thin strap-like axes for *A. nodosum*). The aim of this study was to examine the distribution of As_{TOT} and iAs within *L. digitata* and *A. nodosum* thalli sampled in uncontaminated watersusing high performance liquid chromatography – inductively coupled plasma mass spectrometry (HPLC-ICP-MS).

2. Materials and methods

2.1 Chemicals

All chemicals used were of analytical grade or higher. 18 MΩ cm water (MilliQ) was used throughout (MilliQ, Millipore, UK). Ammonium carbonate ($(NH_4)_2CO_3$) (p.a., BDH), concentrated nitric acid (70 % HNO_3, p.a., Fisher, UK) and 30 % H_2O_2 (p.a., Fisher UK) were used. Arsenate stock solution (1000 mg kg^{-1}) was from High Purity Standards (USA), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) were from ChemService (USA) and AccuTrace® ICP-MS 2 (10 mg kg^{-1} As) from AccuStandard (USA) was used to quantify As_{TOT}. Certified reference materials used were BCR-211 (rice) certified for iAs and ERM-CD200 (*Fucus vesiculosus*), certified for As_{TOT}, both from IRMM (Belgium).

2.2 Study sites and sampling

Sampling was conducted in the inter-tidal zone of a rocky shoreline at Spiddal, Galway Bay, on the west coast of Ireland (53.2409 N, -9.3095 W) in October 2015 (Figure S1). Routine monitoring by the Marine Institute as part of the national programme for coastal water shows Galway Bay as a relatively unpolluted bay, with arsenic levels in seawater and biota typical of other locations on the west coast of Ireland (Figure S2). Growing whole thalli (n=5) of *Laminaria digitata* were harvested at low tide from the upper limit of their distribution in the intertidal zone by cutting the stipe at the holdfast.

The main axis of 25 *Ascophyllum nodosum* (excluding laterals) which were free of visible epiphytes and had little or no physical or herbivore-induced damage were cut from growing fronds. Salinity, water temperature, dissolved oxygen and pH were measured *in situ* during sample collection with a YSI probe (ProDSS, Yellow Springs, USA) on 02 October 2015. They were 29.8 PSU, 14.5°C, 8.55 mg/L and pH 8.21, respectively.

2.3 Sample preparation

Algal samples were transported to the laboratory in cool conditions and rinsed under deionised water. Potential microscopic epiphytes and biofilms were removed by scraping the thallus surface with a PTFE spatula. The length and weight of each thallus was recorded. Five thalli of *L. digitata* were treated individually and sub-divided into stipe, meristem, young blade, middle blade and decaying distal blade as shown in Figure 1. For each thallus, the middle blade sections were pooled, as were the distal blade sections and decaying distal blade sections, resulting in six thallus parts per thallus, a total of 30 samples.

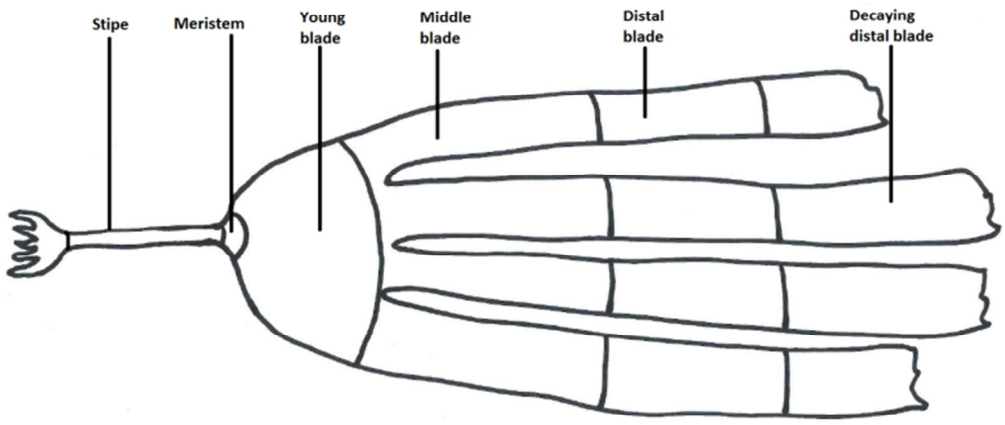

Figure 1: Sub-sampling sections as taken from a *Laminaria digitata* thallus.

131 *A. nodosum* thalli (n = 25) were divided into annual growth segments (or internodes) according to
132 location of air bladders as per Stengel et al. (2005) and pooled into samples consisting of 5 fronds per
133 annual segment in order to obtain sufficient biomass for analysis. Each sample was cut into
134 approximately 2 x 2 cm^2 or 2 cm lengths, as appropriate, and frozen at - 20°C prior to lyophilisation in
135 a Labconco Freezone freeze-dry system and bulk tray drier (Labconco Corporation, Kansas City,
136 USA). Samples were homogenised to a fine powder with a Knife Mill Grindomix GM200 Miller
137 (Retsch, GmbH). Sample extraction and analysis was completed at the Trace Element Speciation
138 Laboratory (TESLA) at the University of Aberdeen.
139
140 2.4 Sample extraction and analysis for iAs (as AsV).
141 0.100 g of freeze-dried algae was microwave extracted with 10 mL of a solution of 1 % (v/v) HNO_3
142 and 2 % H_2O_2 in order to oxidise AsIII to AsV. The microwave was a Mars 5 non-pressurised system
143 with a temperature programme of 5 minutes at 50°C, 5 minutes at 75°C and 30 minutes at 95°C. The
144 extracts were centrifuged (4500 rpm) and the supernatant removed for analysis. The limit of detection
145 and quantification (mg kg^{-1} sample) were 0.001 and 0.01, respectively. To estimate total extracted
146 arsenic, an aliquot of the supernatant was diluted 1:5 with 1% HNO_3 as detailed in section 2.5.
147
148 Speciation analysis was conducted using an Agilent 1260 HPLC coupled to an Agilent 8800 ICP-MS.
149 A 4.1 x 250 mm Hamilton PRP-X100 column (Hamilton Co., Reno, /NV, USA) was used for
150 chromatographic separation. The mobile phase was 30 mM $(NH_4)_2CO_3$ at pH 8.5. The column outlet
151 was directly coupled to the ICP-MS via a T-piece. Germanium was used as internal standard, added
152 via a T-piece. The arsenic content was calibrated with a DMA standard for iAs and all other As
153 species, since the As response is species independent and the As response did not change within the
154 chromatographic run. The instrument was operated in standard mode using MS/MS mode with
155 oxygen as reaction gas (^{75}As-> ^{91}AsO) and As was determined in the mass shift mode as AsO^+ on m/z
156 91, while the first MS was set on m/z 75 for As in order to prevent interference from $ArCl^+$.
157
158 2.5 Sample extraction and analysis for As_{TOT}

159 0.100 g of freeze-dried algae was pre-digested overnight in 2 mL of concentrated HNO_3. It was then
160 digested using an Ethos Up microwave system (UK) with inserts with the following temperature
161 programme: room temperature to 200°C in 15 minutes, hold at 200°C for 15 minutes, then allowed to
162 cool. The digest was then transferred to a 50 mL tube and diluted to 50 mL with deionised water.
163 AccuTrace diluted with 1 % HNO_3 was used as external standard.
164
165 ICP-MS analysis which was conducted on an Agilent 8800 ICP-MS in MS/MS mode with O_2 as
166 reaction gas and As was measured in the mass-shift mode on m/z 91 (AsO^+). Germanium as internal
167 standard was added via a T-piece. External calibration was conducted using Accutrace diluted with
168 1% HNO_3. The limit of detection and quantification (mg kg^{-1} dry weight sample) were 0.002 and 0.02,
169 respectively based on three and ten times the standard deviation of the blank level of the extract using
170 the average sample to solvent ratio.
171 2.6 Verification of iAs as AsV and the absence of As-sugars using HPLC-ICP-MS/ESI-MS.
172 Verification of AsV and the absence of As-sugars especially As-sugSO_4 which might co-elute with
173 AsV, was completed on a sub-set of *L. digitata* samples using HPLC- ICP-MS and simultaneously
174 ESI-MS (Maxis II, Bruker, Germany). A splitter was attached to outlet of the HPLC column (as for
175 HPLC-ICP-MS). The split ratio was 1:3 ICP-MS / ESI-MS, with a flow rate of 1 mL/min. The
176 injection volume was 50 µL. The ESI-MS was operated in positive mode, with a capillary voltage of
177 4.5 kV, 8 Hz for MS, MS/MS between 1 & 4 Hz depending on sensitivity. MS/MS was used in auto-
178 MS/MS mode with preference given to the m/z ratios of As-sugars.
179
180 2.7 Statistical analysis
181 Minitab 17 software (Minitab Ltd., Coventry, UK) was used for statistical analysis. For all data sets,
182 tests for normal distribution were conducted using a Hartley's F-max test. For normally distributed
183 data, a one way analysis of variance (ANOVA) was performed followed by a Tukey HSD post-hoc
184 test. A Kruskal-Wallis test was performed where data were not normally distributed. The data were
185 tested for significant differences in concentrations of As_{TOT} and iAs between the species, within each

species sampled (between thalli) and between thallus parts for *L. digitata* and between different years for *A. nodosum*.

2.8 Quality assurance

Method blanks and certified reference materials (CRM: BCR-211 (rice) and ERM-CD200 (*Fucus vesiculosus*) were treated in the same manner as samples. Recovery of As_{TOT} in BCR-211 (n = 2) was 90.4 % ± 5.7 % and ERM-CD200 (n = 1) was 96.4 %. Recovery of iAs in BCR-211 was 97.6 % thus confirming the suitability of the method.

2.9 Confirmation of detection of iAs

The unequivocal determination of iAs as AsV in *L. digitata* is supported by the extraction technique used, the chromatographic separation with ICP-MS and the simultaneous specific detection of As-sugars using their protonated molecular masses (ESI-MS). The acid extracted sample (Figure S3) clearly shows the presence of DMA and the aglycone-free As-sug resulting from the breakdown of the sidechain from the other sugars during extraction (Gamble et al., 2002). The same sample but extracted using water only (Figure S4) clearly shows the presence of DMA, As-Sug-OH, As-Sug-PO_4 and As-Sug-SO_3. While As-Sug-SO_4 is present only at trace levels and therefore cannot be considered as influencing the AsV signal, it could interfere if present in other samples at a higher concentration and only a water extraction was used. These measurements clearly show that all As-sugars lose their side chain during acid extraction to the aglycone-free sugar (As-sug) but do not disintegrate to iAs. Hence the acid extraction was used for samples in this study. As-sug was not present in the water extract but represents all As-sugars which lose their side-chain during the acid extraction and it did not interfere with the determination of iAs in the used HPLC-ICP-MS method.

3. Results

3.1 Total As distribution in *Ascophyllum nodosum* and *Laminaria digitata*

Results are shown in Figure 2. In different *A. nodosum* growth sections, As_{TOT} ranged from 38 to 111 mg kg^{-1} dry weight (DW), with an increasing trend with age of the thallus part, and highest

concentrations were detected in the 5-year old parts (111 mg kg^{-1} DW). However, these differences in As_{TOT} concentrations were not significant ($p = 0.178$, n = 5 samples per part) due to the large variance in the measured As concentrations. There were significant differences in As_{TOT} between the thalli, with one sample containing significantly higher levels than two others ($p < 0.01$), but it was not significantly higher than the remaining two ($p > 0.05$). For detailed results, see tables S1 and S2 in the supporting information.

In *L. digitata* thallus parts, As_{TOT} ranged from 36 to 131 mg kg^{-1} DW (tables S1 and S2), with the highest concentrations detected in the distal (131 mg kg^{-1} DW) and decaying distal blade sections (129 mg kg^{-1} DW). There were no significant differences in As_{TOT} concentrations between the parts (one way ANOVA, $p = 0.169$, n = 5 thallus subsamples per part). There were no significant differences in As_{TOT} between the thalli (Kruskal-Wallis $p = 0.181$). Concentrations of As_{TOT} were significantly higher in *L. digitata* than in *A. nodosum* ($p = 0.026$).

3.2 Inorganic As (as AsV) distribution in *Ascophyllum nodosum* and *Laminaria digitata*

Inorganic arsenic was detected in all *A. nodosum* samples, and comprised less than 1% of the total arsenic. iAs concentrations did not differ significantly between the yearly sections (Kruskal-Wallis, $p = 0.997$), but concentrations ranged from 0.007 to 0.070 mg kg^{-1} DW; For one sample, two older sections aged 4 and 5 years, were considerably higher at 0.178 and 0.703 mg kg^{-1} DW, respectively. These two sections also had the highest As_{TOT} concentrations (98 and 111 mg kg^{-1} DW, respectively. When treated as whole thalli (years 1 to 5 for each sample), there were significant differences in iAs concentrations between the samples ($p = 0.009$, Kruskal Wallis), with two samples containing significantly higher iAs concentrations than the three others.

iAs was detected in all *L. digitata* samples, increasing with distance from the stipe towards the distal parts (Figure 2). Concentrations of iAs were significantly higher in *L. digitata* than in *A. nodosum* ($p < 0.01$) ranging from 2.2 to 87 mg kg^{-1} DW in *L. digitata*. There were no significant differences iAs between the thalli (Kruskal-Wallis $p = 0.774$), but there were significant differences between the parts

(one-way ANOVA p = 0.0009). iAs concentrations in the stipe, meristem, and young blade were significantly lower than concentrations in the middle, distal and decaying distal blades (one way ANOVA, $p < 0.05$). There were no significant differences in concentrations between the stipe, meristem and young blade (p = 0.071) or between the middle, distal and decaying distal blades (p= 0.052). Concentrations in the stipe were significantly lower than those in the distal blades (p = 0.009) and decaying distal blade (p = 0.009). Concentrations in the meristem were also significantly lower than those in the distal blades (p = 0.020) and decaying distal blades (p = 0.021). When expressed as a percentage of As_{TOT}, iAs comprised more than 50 % of the total arsenic content in most of the middle, distal and decaying distal blades, but it was always less than 50% of the As_{TOT} concentration in the stipe, meristem and young blade in *L. digitata*

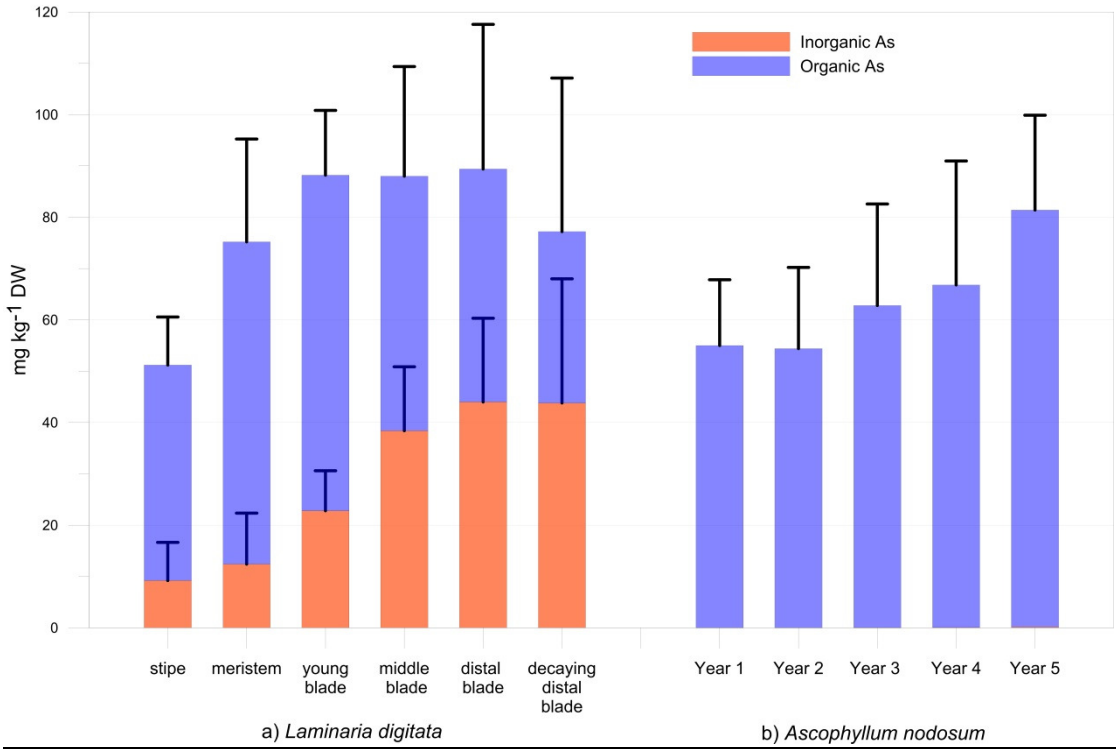

Figure 2. Mean concentrations of total arsenic (error bar one standard deviation, n=5) and concentrations present as inorganic arsenic (red, error bar one standard deviation, n=5) for *Laminaria digitata* (a) and *Ascophyllum nodosum* (b)

259

260 **4. Discussion**

261 Seaweeds analysed in this study were derived from a mixed water environment on the northern shore
262 of Galway Bay in western Ireland. Routine monitoring of arsenic in seawater and shellfish from
263 Galway Bay shows arsenic concentrations that are not elevated and therefore the sampling site can be
264 considered to represent a relatively uncontaminated site with respect to arsenic (Figure S2). The
265 calculated mean concentrations of As_{TOT} in *Laminaria digitata* and in *Ascophyllum nodosum* thalli
266 were 78 ± 24 and 64 ± 20 mg kg^{-1}, respectively. A. nodosum is the primary seaweed harvested in
267 Ireland with agriculture/horticulture representing the major sector of application. The current EU limit
268 for As in animal food and feed products is 40 mg kg^{-1} (12 % moisture), and iAs must be less than 2
269 ppm. When a factor of 12 % moisture was applied to results in this study, the mean As_{TOT}
270 concentrations in both species were above this limit at 69 and 57 mg kg^{-1}, respectively.
271
272 The highest iAs content was 87 mg kg^{-1}, recorded in decaying distal tips of *L. digitata.* While iAs in
273 *A. nodosum* was consistently below 1 % of As_{TOT}, iAs in *L.digitata* accounted for more than half of
274 As_{TOT}, particularly from the middle of the blade to the distal tips. There was a subtle increase in As_{TOT}
275 and iAs with thallus section age in *A.nodosum*, although this was not statistically significant. Mean
276 concentrations of iAs in all thallus parts for *L. digitata* exceeded both the French limit of 3 mg kg^{-1}
277 DW for seaweed as food, and the limit of 1 mg kg^{-1} in seaweed set by the Australia New Zealand
278 Food Standards Authority, typically by an order of magnitude in the young, middle, distal and
279 decaying distal blade.
280
281 Concentrations of As_{TOT} and iAs in *L. digitata* and *A. nodosum* measured in this study are similar to
282 those detected by Taylor and Jackson (2016), who found As_{TOT} ranging between 50 and 106 mg kg^{-1},
283 and 2.8 to 20 mg kg^{-1} iAs in *L. digitata*, and As_{TOT} between 23.1 and 23.7 mg kg^{-1} and iAs between
284 0.06 and 0.08 mg kg^{-1} iAs in *A. nodosum* from New England, USA. Relatively high and variable
285 concentrations of As_{TOT} in *L. digitata* have also been reported in some previous studies (see Table 1).
286 Maulvault et al. (2015) found iAs comprised approximately half and <1% of AS_{TOT} in *L. digitata* and

287 *S. latissima* respectively in a Norwegian fjord, despite similar As_{TOT} concentrations of *ca*. 40 mg kg^{-1}

288 [1]. However other studies have reported iAs as "not detected" in *L digitata* .

289

290 Table 1: Comparison of the As_{TOT} and iAs concentrations determined in *L. digitata* in this study with

291 levels reported in various other studies (mg kg^{-1} DW)

Location	As_{TOT}	iAs	Reference
Ireland	59 – 114[a]	30 – 62[a]	This study
New England, USA	50 - 106	2.8 - 20	Taylor and Jackson, 2016
Norway	64		Mæhre et al., 2014
Norway	73 - 107	0.1 - 7.7	Duinker, 2014
Ireland	49.4 - 89.6		Ratcliff et al., 2016
France	93 ± 2	77 ± 3 AsV	García-Salgado et al., 2012
Spain (Market)		ND <2 ng g^{-1}	García Salgado et al., 2006
Scotland		ND[b]	Feldmann et al., 2000
Norway	41 ± 19	20 ± 1	Maulvault et . al 2016

292 a range of concentrations determined in the blades for five thalli (stipe and meristem excluded); b extraction was methanol/water 1:1 and iAs maybe co-
293 eluted with arsenosugar.
294
295

296 Metal concentrations in macroalgae have previously been shown to vary between species and thallus
297 parts. For example, Stengel et al. (2005) found distinctly different patterns in the distribution of Cu,
298 Fe and Mn in stipe and blade sections of *L. digitata,* with Fe lowest in the meristem and young blade,
299 Mn higher in the distal blades than in the stipes, and Cu highest in the meristem and young blade.
300 These differences were likely due to growth pattern and tissue function. The authors also found
301 considerable differences in tissue Cu, Mn and Fe concentrations between functional thallus parts of
302 *A.nodosum* from the same site, with Fe and Cu increasing with increased age of the thallus part.
303 Similary, metal-specific differences in concentrations of Fe, Cu, Cr, Zn, Mn, Pb Cd, Ni and Al were
304 also found in different thallus parts of the kelp *Lessonia trabeculata* (Sáez et al., 2012). Variation in
305 differences may be site-specific, related to differing growth rates, and affected by environmental
306 factors such as light, temperature, nutrients and salinity (Duinker et al., 2016; Stengel et al., 2005,
307 Stengel et al., 2011). In *A. nodosum*, the tips are the youngest part, in contrast to *L. digitata,* where the
308 meristematic sections are the young actively growing sections, and the distal tips are the oldest part.

309 However this difference in growth pattern is not sufficient to explain the observed differences in iAs
310 concentration. The specific binding mechanisms of metals in brown algae are currently poorly
311 understood (Connan and Stengel, 2011a, 2011b); similarly the reasons for the difference in iAs
312 content between *A. nodosum* and *L. digitata*, as well as within-algal differentiation and potential
313 mechanisms of conversion due to metabolic processes within different thallus regions require further
314 investigation.
315
316 Low levels of iAs and organic arsenic have been measured in most foodstuffs, with typical
317 concentrations of iAs less than 0.25 mg kg^{-1}, however, certain foods such as rice and seafood may, in
318 some instances, contain considerably higher levels. The EFSA Comprehensive European Food
319 Consumption Database estimates that mean dietary exposure among the adult population ranged from
320 0.09 to 0.38 μg kg^{-1} iAs body weight per day, with grain based processed products (non rice-based)
321 the main contributor (EFSA, 2014). European Commission Regulation (EC) No 1881/2006 currently
322 only sets the maximum limit for iAs for certain rice products for children and infants, although
323 maximum limits for other foodstuffs, including seaweed and seaweed-based products, may be
324 included in future regulations. EFSA reported Hijiki (*Hijiki fusiforme*, Fucales, Phaeophyceae*)*
325 seaweed containing 11 mg kg^{-1} iAs, which was estimated as correlating to an exposure of 1.57 μg kg^{-1}
326 per day for chronic consumption (10 g per day) (EFSA, 2014), although this reported concentration is
327 considerably lower than the value of 96 mg kg^{-1} reported by Rose et al. (2007), and 117 mg kg^{-1},
328 reported by Almela et al. (2006). Despite the high iAs concentrations in Hijiki products, EFSA did not
329 consider it to be an important source of iAs exposure in that study due to the low consumption rate of
330 Hijiki in the European population and a similar conclusion might be expected for *L. digitata*. In recent
331 years, consumption of algae and their use in dietary supplements has grown in popularity. Although,
332 there are currently no EU limits on iAs in seaweed for human consumption, in 2010 the U.K. Food
333 Standards Agency recommended that the consumption of *Hizikia fusiforme* be avoided due to high
334 levels of iAs (FSA, 2010) and, in 2015, the Superior Health Council of Belgium recommended that
335 consumption of *Hijiki* should be avoided (SHC, 2015). It is now clear that seaweed species other than
336 Hijiki can also contain elevated and variable concentrations of iAs. The small dataset available, and

variable iAs concentrations observed for certain seaweed species presents a challenge for regulators and food safety authorities in setting maximum limits for seaweed and in providing specific consumer advice. There are also challenges for the seaweed industry to provide data demonstrating product quality, and the complexity of the iAs analysis, which generally employs hyphenated techniques such as HPLC-ICPMS, may be a barrier in this regard. New field deployable techniques offer potential for lower cost screening of iAs in seaweeds and this may aid in the assessment and management of risks associated (Bratelei et al. 2017).

While the concentration of As_{TOT} detected in *A. nodosum* in this study was frequently above the EU limit for As_{TOT} in seaweed meal and feed materials derived from seaweed, it was predominantly organic arsenic, and the iAs content was consistently less than 1% of the As_{TOT} content and always lower than 1 mg/kg, thus satisfying the maximum limits for seaweed as food in France and Australia. While, the toxicity of organoarsenicals is not well characterized, it is considered to be much lower than that of inorganic arsenic (iAs) (Francesconi and Edmonds, 1998, Feldmann and Krupp, 2011), suggesting a much lower to negligible risk from utilising *A. nodosum* than *L. digitata* in agri/food and health applications. Further work is required to better characterise the risk to consumers associated with iAs in seaweeds, including *L. digitata*, and to understand the factors influencing the presence of different As species. Additionally, data on potential seasonal and spatial variation in iAs content in commercially valuable seaweed species are required to implement adequate protocols regarding algal harvesting and processing. These will aid food safety agencies in evaluating and managing risks to consumers and assist the development of safe and sustainable commercial seaweed utilization in the future

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482

List of Figures

Figure 1: Sub-sampling sections as taken from a *Laminaria digitata* thallus.

Figure 2. Mean concentrations of total arsenic (error bar one standard deviation, n=5) and concentrations present as inorganic arsenic (red, error bar one standard deviation, n=5) for *Laminaria digitata* (a) and *Ascophyllum nodosum* (b)

Supplementary information

Figure S1: Location of water sampling sites in Galway Bay (triangles) and Spiddal seaweed collection site (green square).

Figure S2: Dissolved total arsenic concentration in seawater ($\mu g\ L^{-1}$) plotted against salinity (PSU) sampled from estuarine, coastal and shelf water around Ireland (blue) and at sampling sites in Galway Bay as shown in Fig S1 (red). The latter are close to the seaweed sampling location This monitoring was undertaken between 2011 and 2015 as part of the Marine Institute's national surveillance and shellfish waters monitoring programme. There is a positive relationship between total dissolved arsenic concentrations and salinity, with increasing dissolved total arsenic concentrations towards the outer bay. Water samples collected in Galway Bay fall within the envelope of Irish coastal and shelf waters indicating concentrations are typical of uncontaminated coastal-shelf waters found on the Irish west coast. Extensive monitoring of arsenic in bivalve molluscs in Galway Bay and around the coast also support this (not shown)

Figure S3: Acid extract of Laminaria digitata distal blade using HPLC- ICP-MS/ESI-MS showing conversion of As sugars to the aglycone-free sugar (As-Sug, in red). Arbitrary intensity of ESI-MS (left y-axis) for DMA on m/z 140, As-sug (m/z 255), As-OH (m/z 329), As-sug-PO4 (m/z 483), As-Sug-SO3 (m/z 393) and As-sug-SO4 (m/z 409) and of ICP-MS for As as AsO+ (m/z 91) (right y-axis).

Figure S4: Water extract of Laminaria digitata distal blade using HPLC- ICP-MS / ESI-MS with sug-SO4 signal multiplied by ten to highlight overlap with AsV peak. Arbitrary intensity of ESI-MS (left y-axis) and of ICP-MS (right y-axis). The monitored m/z are the same as in Figure S3.

List of Tables

Table 1: Comparison of the As_{TOT} and iAs concentrations determined in *L. digitata* in this study with levels reported in various other studies (mg kg^{-1} DW)

Location	As_{TOT}	iAs	Reference
Ireland	59 – 114[a]	30 – 62[a]	This study
New England, USA	50 - 106	2.8 - 20	Taylor and Jackson, 2016
Norway	64		Mæhre et al., 2014
Norway	73 - 107	0.1 - 7.7	Duinker, 2014
Ireland	49.4 - 89.6		Ratcliff et al., 2016
France	93 ± 2	77 ± 3 AsV	García-Salgado et al., 2012
Spain (Market)		ND <2 ng g^{-1}	García Salgado et al., 2006
Scotland		ND[b]	Feldmann et al., 2000
Norway	41 ± 19	20 ± 1	Maulvault et . al 2016

a range of concentrations determined in the blades for five thalli (stipe and meristem excluded); b extraction was methanol/water 1:1 and iAs maybe co-eluted with arsenosugar.

Supplementary Information

Table S1: Concentrations of AsTOT and iAs in Laminaria digitata and Ascophyllum nodosum (mg kg-1 Dry Weight)