Intercomparison of Extraction and Analytical Techniques for Inorganic Arsenic in Seaweed

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Arsenic Overview

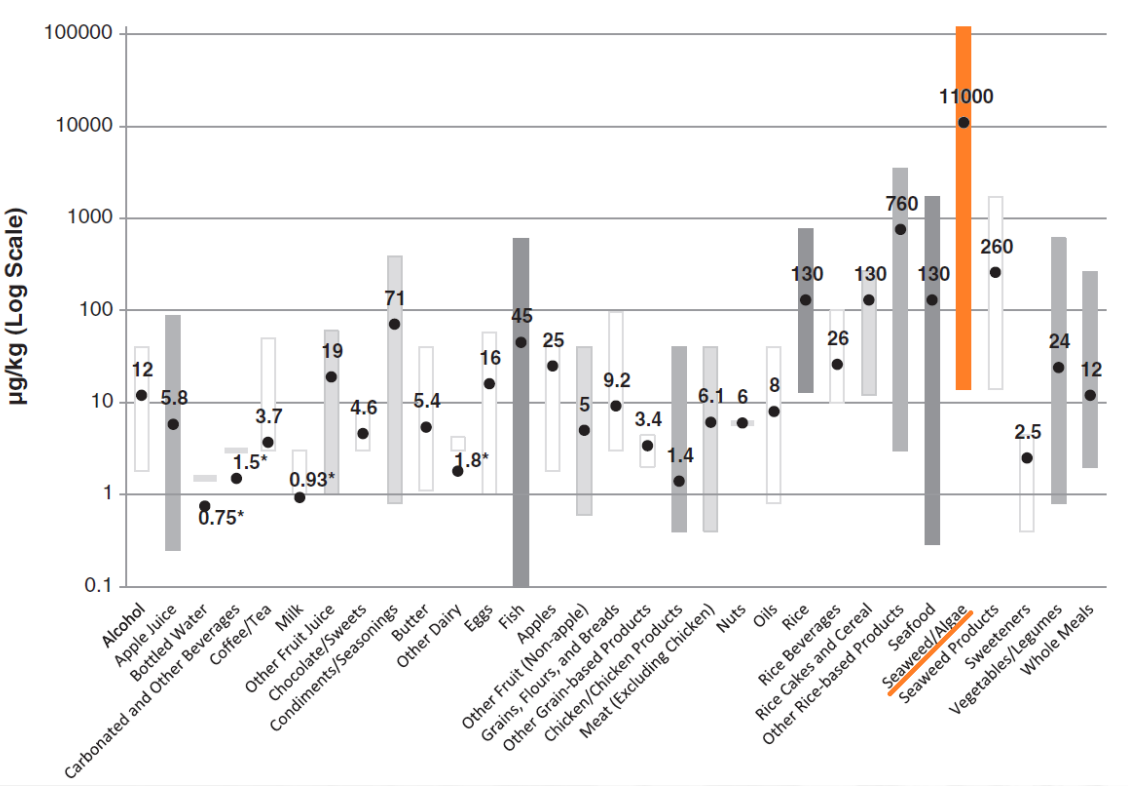
- Ubiquitous in the environment
- Exposure occurs due to both natural and anthropogenic sources
- Health effects due to chronic exposure are known to include cancers, cardiovascular disease, and dermatological issues
  - Some evidence that As exposure can cause diabetes and respiratory, neurological, and immune issues
- Most common exposure route in the US is ingestion via water and food[1]
  - If drinking water source > 10 µg/L, then water is usually the primary source of exposure
  - If drinking water source < 10 µg/L, then diet usually is the primary source
- Toxicity depends on the molecular form (species) of As:
  - As(III) > As(V) > MMAs(V) > DMAs(V)
  - Organic As generally *considered* non-toxic or of low toxicity

[1] Kurzius-Spencer, Margaret et al., *Contribution of Diet to Aggregate Arsenic exposures—An Analysis across Populations,* Journal of exposure science & environmental epidemiology 24.2 (2014): 156–162.

Arsenic Regulations

- Numerous regulations exist for As in the US and abroad
- Regulations involving water have existed for some time:
  - 10 µg/L limit for drinking water (US EPA and WHO)
- Despite food being a major source of As exposure, few regulations exist and most are relatively recent:
  - 10 µg/L inorganic As limit in apple juice (US FDA)
  - 100 ppb inorganic As limit in infant rice cereal (US FDA)
  - 0.2 mg/kg limit for inorganic As in polished rice (UN FAO / WHO)
  - 3 mg/kg limit for inorganic As in algae (France)
  - 1 mg/kg limit for inorganic As in seaweed (Australia / New Zealand)

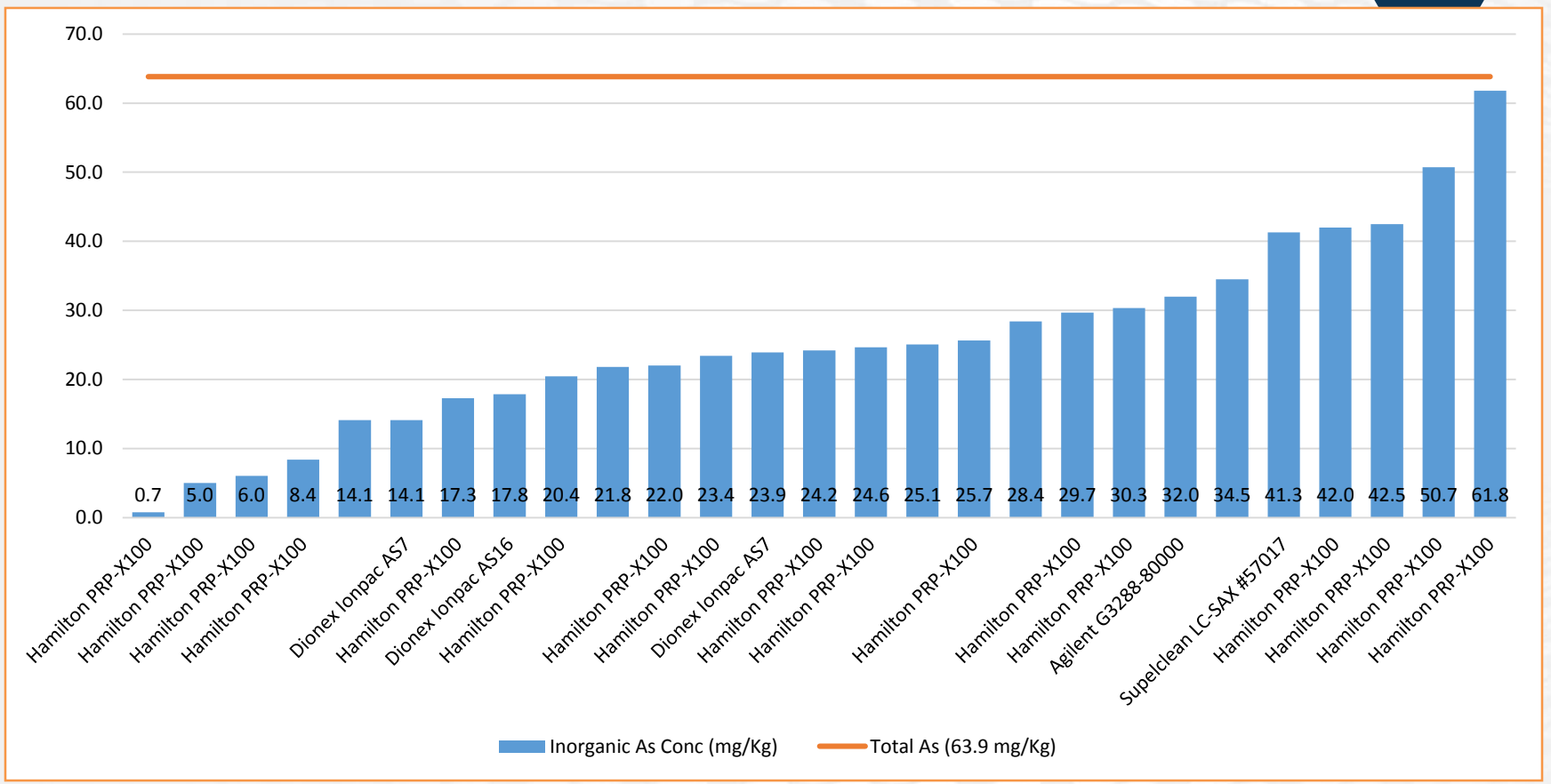
Why Focus on Seaweed?



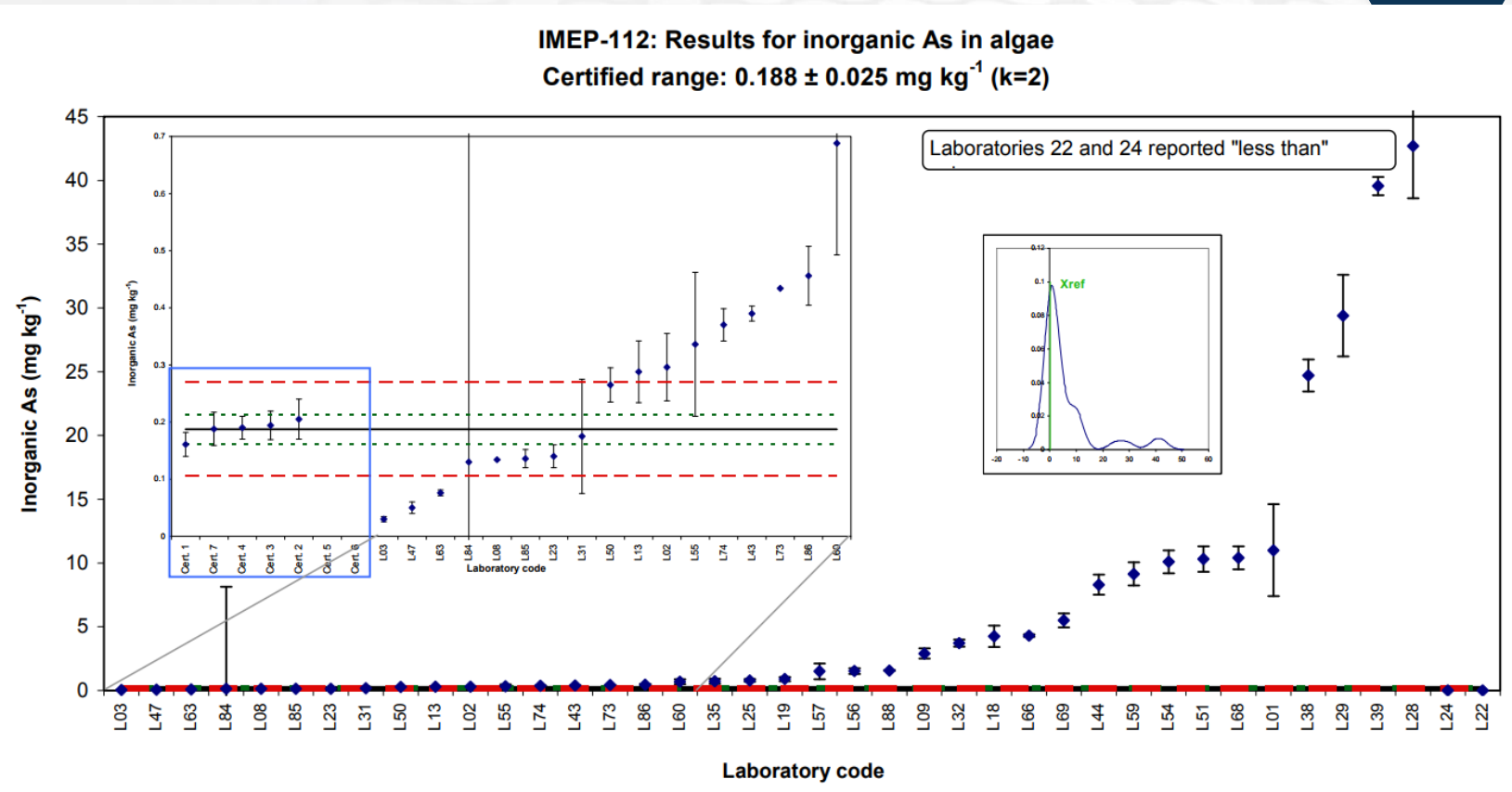
- Generally contain the highest total & inorganic As concentrations among dietary sources
- Inorganic As content varies significantly (low ppb to hundreds of ppm)
- Complex matrix – known to contain numerous As species with varying properties
- Previous studies indicate analytical challenges for accurate & reproducible inorganic As quantitation in seaweed

Adapted from: H. Lynch, et. al., *A comprehensive evaluation of inorganic arsenic in food and considerations for dietary intake analyses*, In Science of The Total Environment, Volume 496, 2014, Pages 299-313.

Brooks Rand Labs Interlaboratory Comparison Study – Kelp (2013)

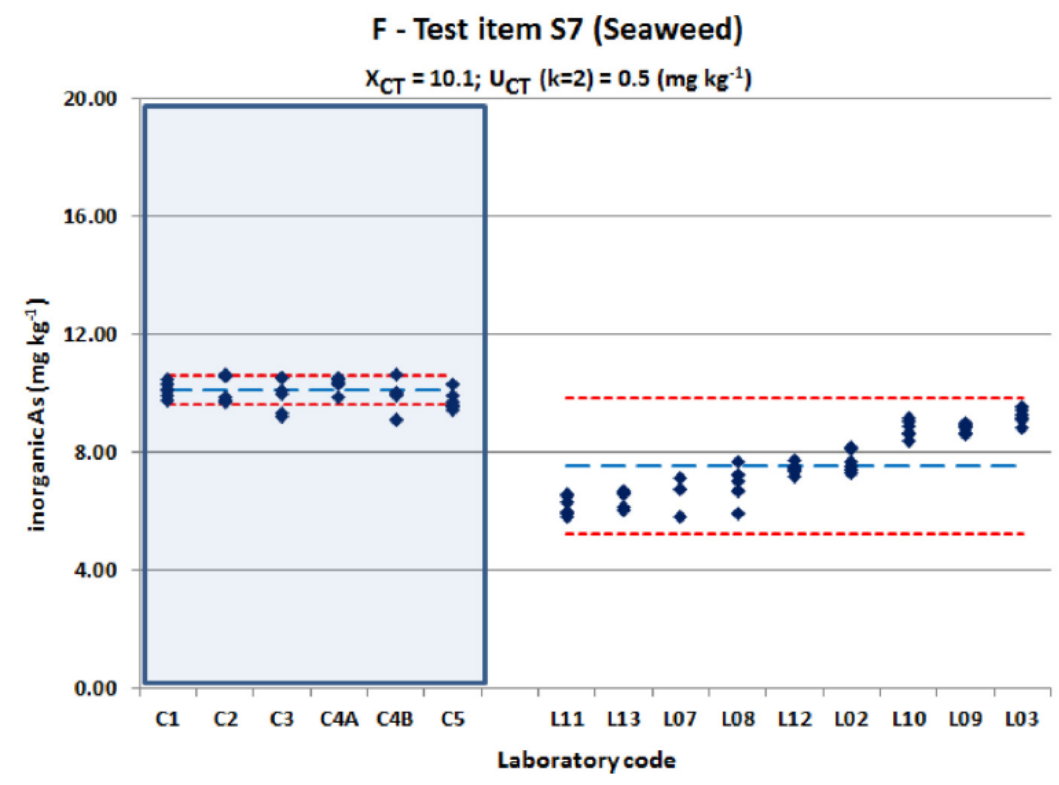


IMEP-112 Inorganic As Results for Algae (2011)



Source: M.B. de la Calle, et al., IMEP-112: *Total and inorganic arsenic in wheat, vegetable food and algae,* Report of the twelfth interlaboratory comparison organised by the European Reference Laboratory for Heavy Metals in Feed and Food, 2011

IMEP-41 Inorganic As Results for NIST NMIJ-7405a *Hijiki* (2014)



Source: I. Fiamegkos, et. al., *Accuracy of a method based on atomic absorption spectrometry to determine inorganic arsenic in food: Outcome of the collaborative trial IMEP-41*, In Food Chemistry, Volume 213, 2016, Pages 169-179.

- Reference labs (C1-C5) used own methods:
  - All extractions involved acid with peroxide
  - Analyses mostly employed LC-ICP-MS
- Participants (L1-L13) all used the same method:
  - HCl digestion, extraction into $CHCl_3$, back-extraction into acid
  - Analysis via FI-HG-AAS

Inorganic As Speciation in Seaweed

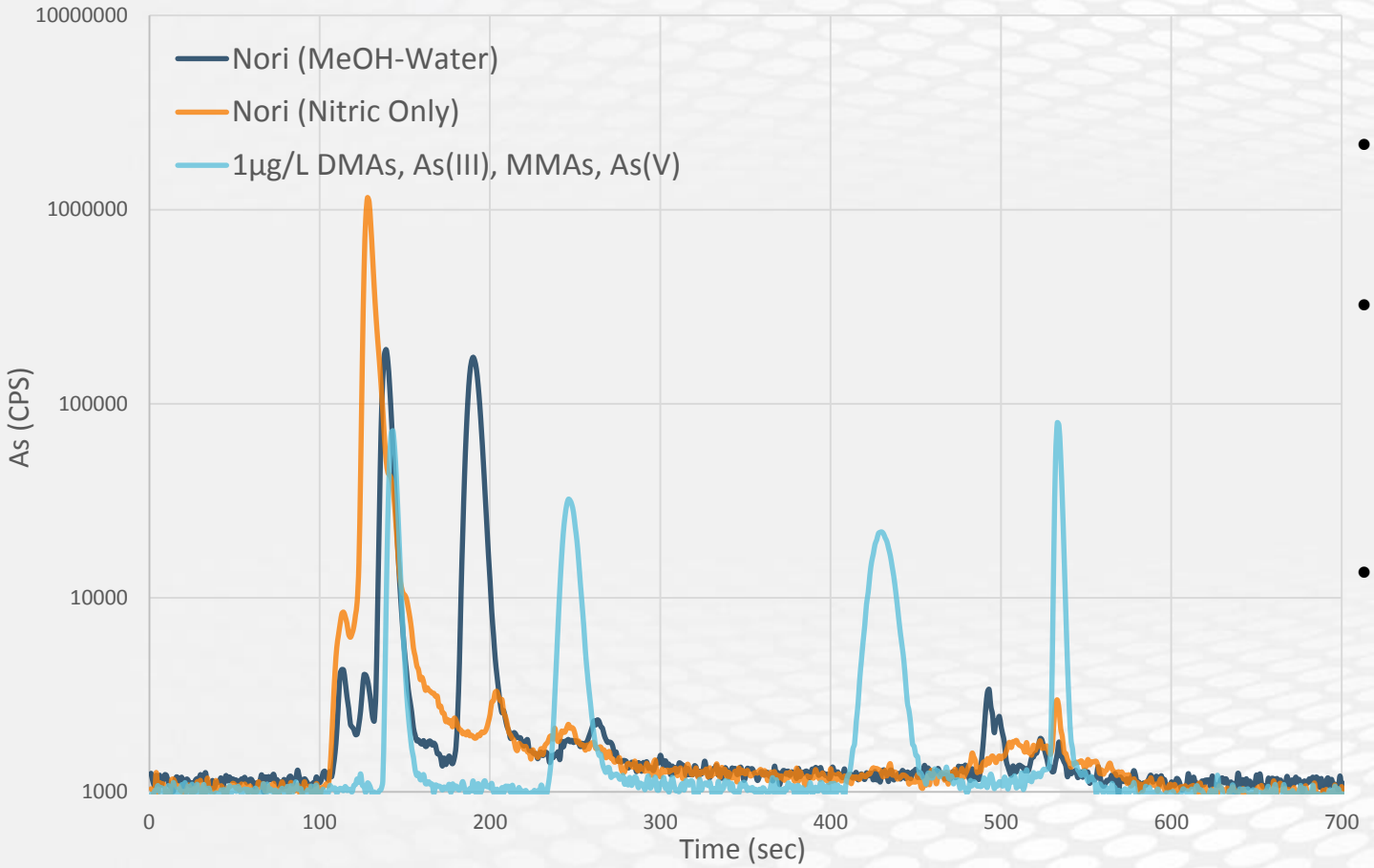
- Typically involves *extraction* of the As species followed by *detection* of the target species of interest
  - Numerous extractions reported in literature, all with limitations
    - Mild extractants (e.g., water or water-methanol mixtures) generally prevent species conversion but may have low extraction efficiencies
      - For example, BAL has observed low arsenate matrix spike recoveries in certain seaweed samples
    - Stronger extractants (e.g., HNO_3) may have higher extraction efficiencies but will generally induce species conversion
      - Cited by many labs who have participated in intercomparison studies (e.g., BAL and IMEP studies)
  - Numerous detection methods have been reported as well:
    - LC-ICP-MS can offer high sensitivity and selectivity, but is generally more expensive and time consuming
    - Selective hydride generation methods are less expensive but may produce false positives for complex matrices[1]

[1]Regmi, R., Milne, B.F. & Feldmann, J. Anal Bioanal Chem (2007) 388: 775

Selected Methodology for this Study

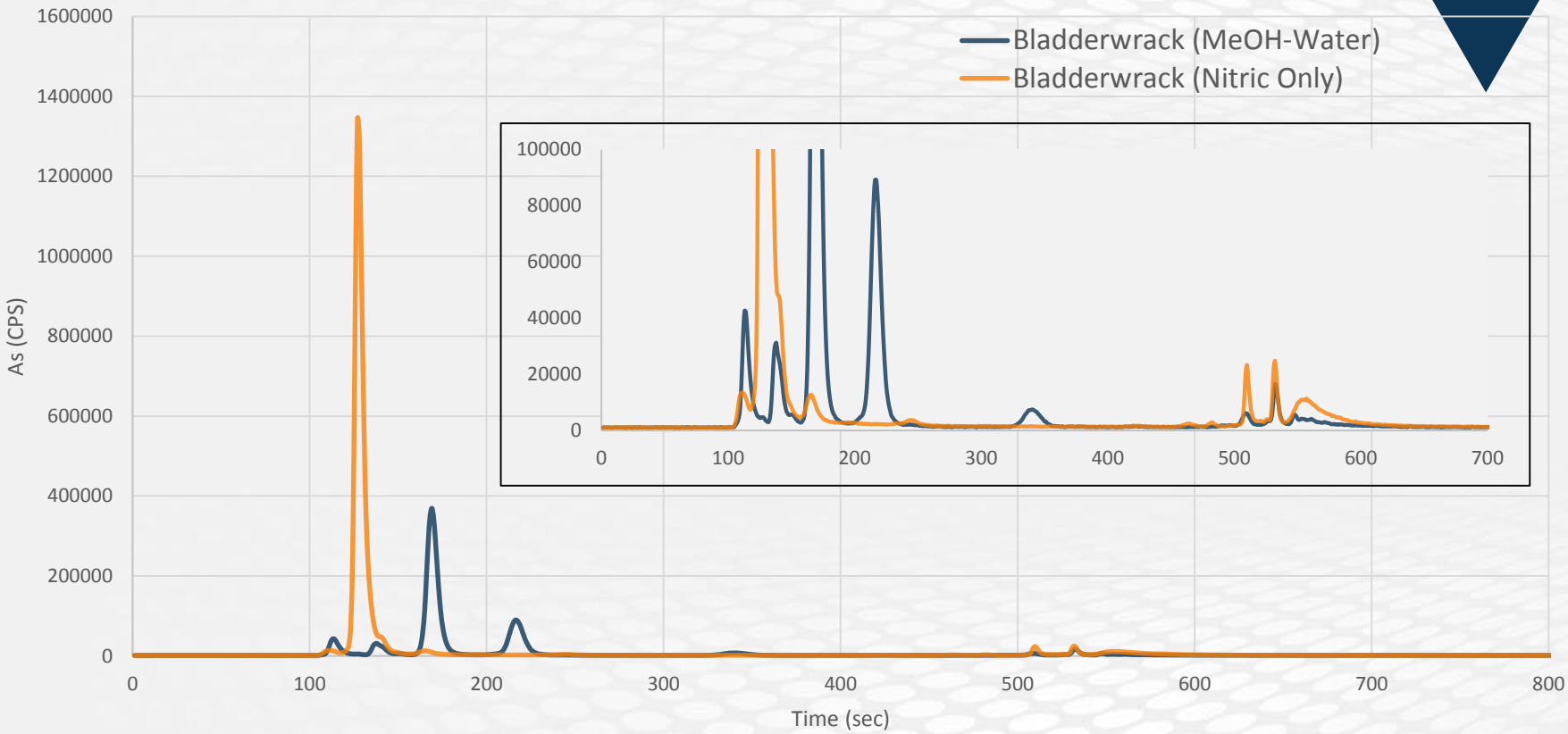
- Total As performed via modified AOAC 2015.01 - microwave digestion (HNO_3 / H_2O_2) followed by ICP-DRC-MS quantitation
- Inorganic As via:
  1. 0.28M HNO_3 extraction, followed by IC-ICP-CRC-MS analysis ("**Nitric Only**")
     - Extraction based on that found in FDA EAM 4.11 for rice and rice-products
  2. Sequential extraction procedure ("**SEP"**) using methanol-water (1:1) *then* 0.28M HNO_3, followed by analysis of both extracts via IC-ICP-CRC-MS
     - Sequential extraction may combine the advantage of mild extractants (i.e., minimal species conversion) with that of more aggressive extractants (i.e., higher extraction efficiencies)
  3. US EPA 1632 - 2M HCl extraction followed by HG-CT-GC-AAS ("**Hydride"**)
- All ICP-MS methods operated in collision or reaction modes to eliminate polyatomic interferences on As
- Chromatography performed using a high capacity anion exchange column
- Analyzed 12 seaweed (macroalgae) samples commercially available in the Seattle area

IC-ICP-CRC-MS Analysis of Extracts

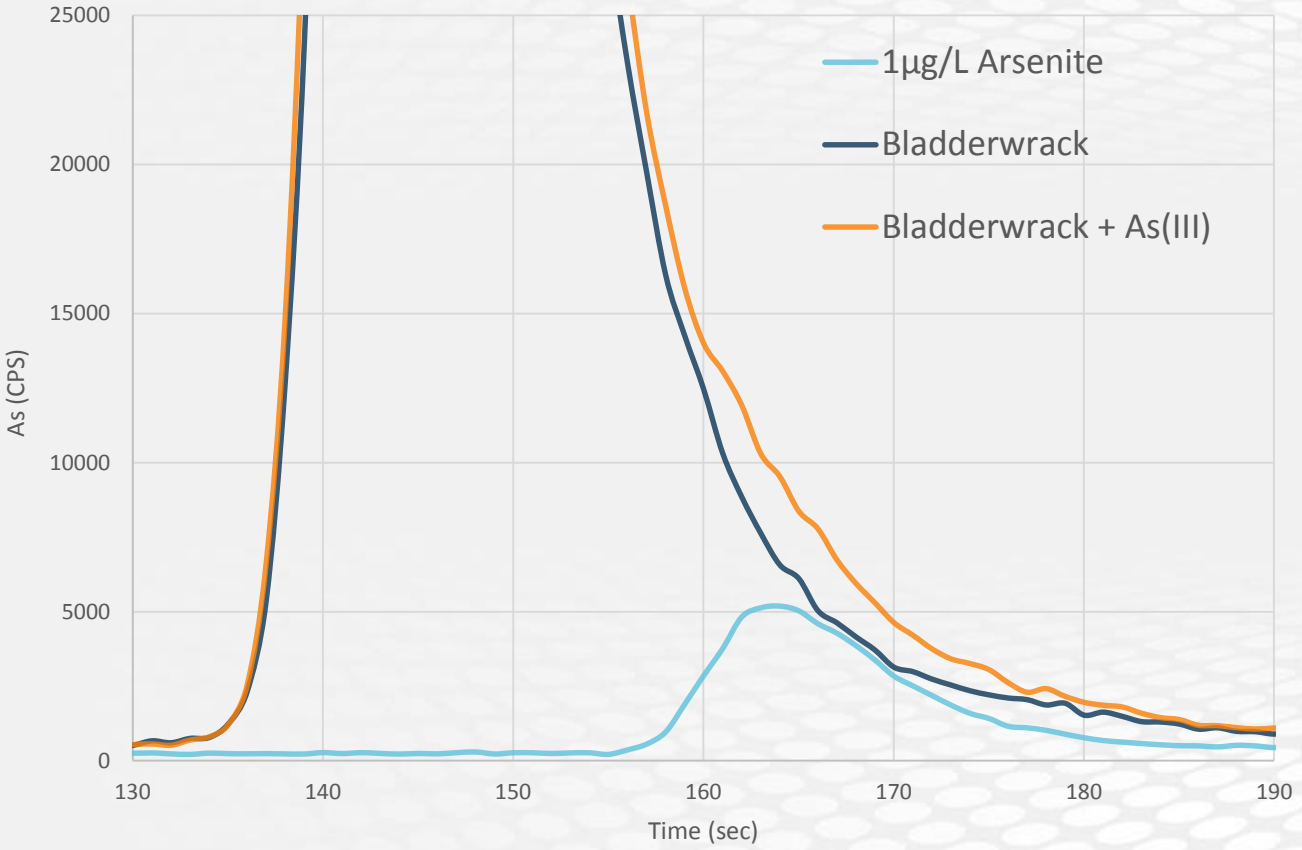


- Arsenosugar peaks observed in MeOH-water extracts
- Nitric extracts contain fewer arsenosugar peaks (note the log scale for As!)
- Degradation of arsenosugars with nitric extracts has been reported extensively in the literature

MeOH-Water vs. Nitric Only – Species Conversion



Importance of Optimized Chromatographic Methods



- Nitric acid extract of Bladderwrack analyzed on PRP-X100 column
- Poor resolution of arsenosugar and As(III)
- Addition of peroxide can convert As(III) to As(V)
  - But oxidation can produce new As species that may interfere with As(III) or As(V)!

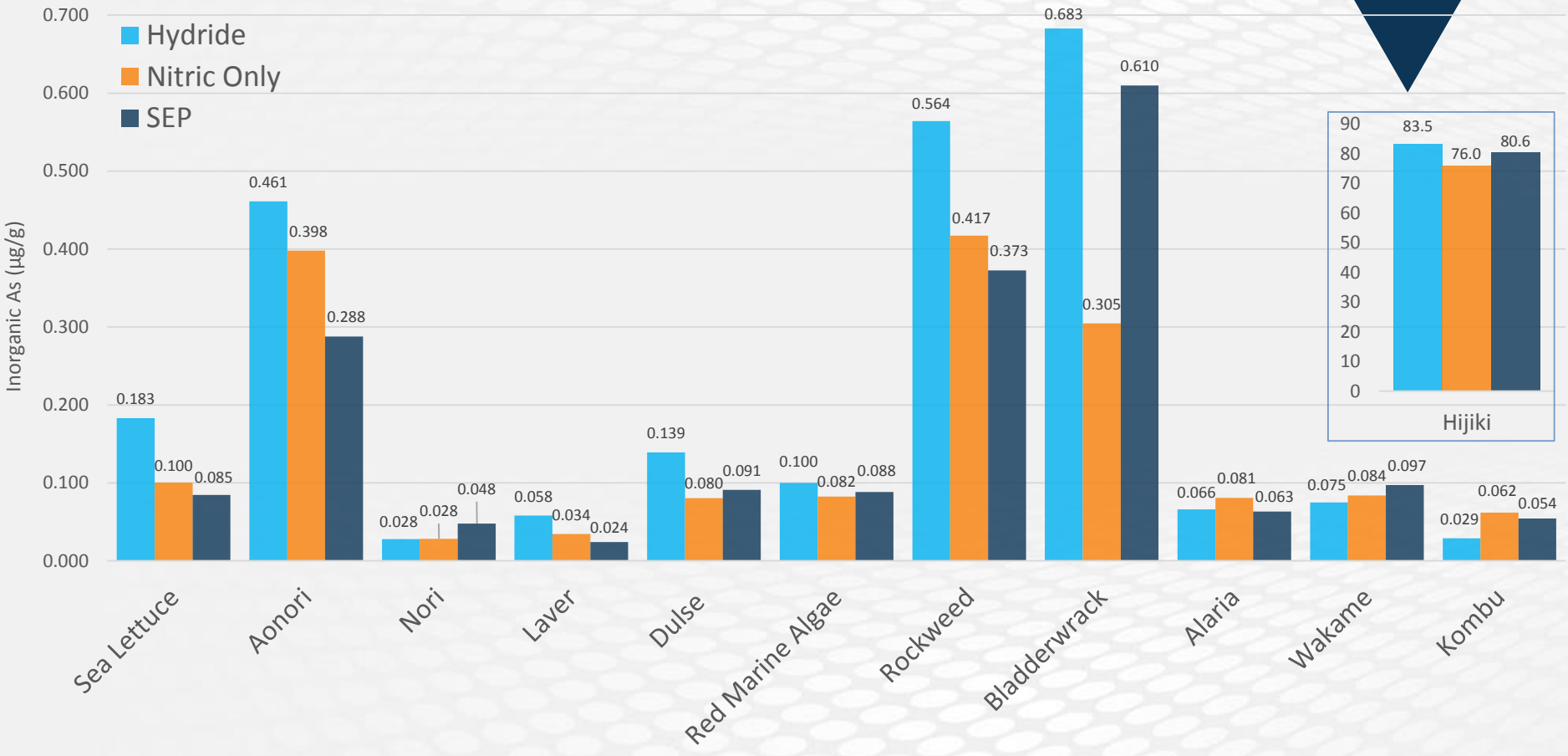
Inorganic As Comparison

Algae Type	Sample Name	Total As	Hydride Inorg. As	Nitric Only Inorg. As	SEP Inorg. As
Green Algae	Sea Lettuce	8.33	0.183	0.100	0.085
Green Algae	Aonori	5.30	0.461	0.398	0.288
Red Algae	Nori	22.8	0.028	0.028	0.048
Red Algae	Laver	27.9	0.058	0.034	0.024
Red Algae	Dulse	13.6	0.139	0.080	0.091
Red Algae	Red Marine Algae	5.88	0.100	0.082	0.088
Brown Algae	Rockweed	27.5	0.564	0.417	0.373
Brown Algae	Bladderwrack	31.7	0.683	0.305	0.610
Brown Algae	Alaria	53.7	0.066	0.081	0.063
Brown Algae	Wakame	42.4	0.075	0.084	0.097
Brown Algae	Kombu	70.7	0.029	0.062	0.054
Brown Algae	Hijiki	93.6	83.5	76.0	80.6

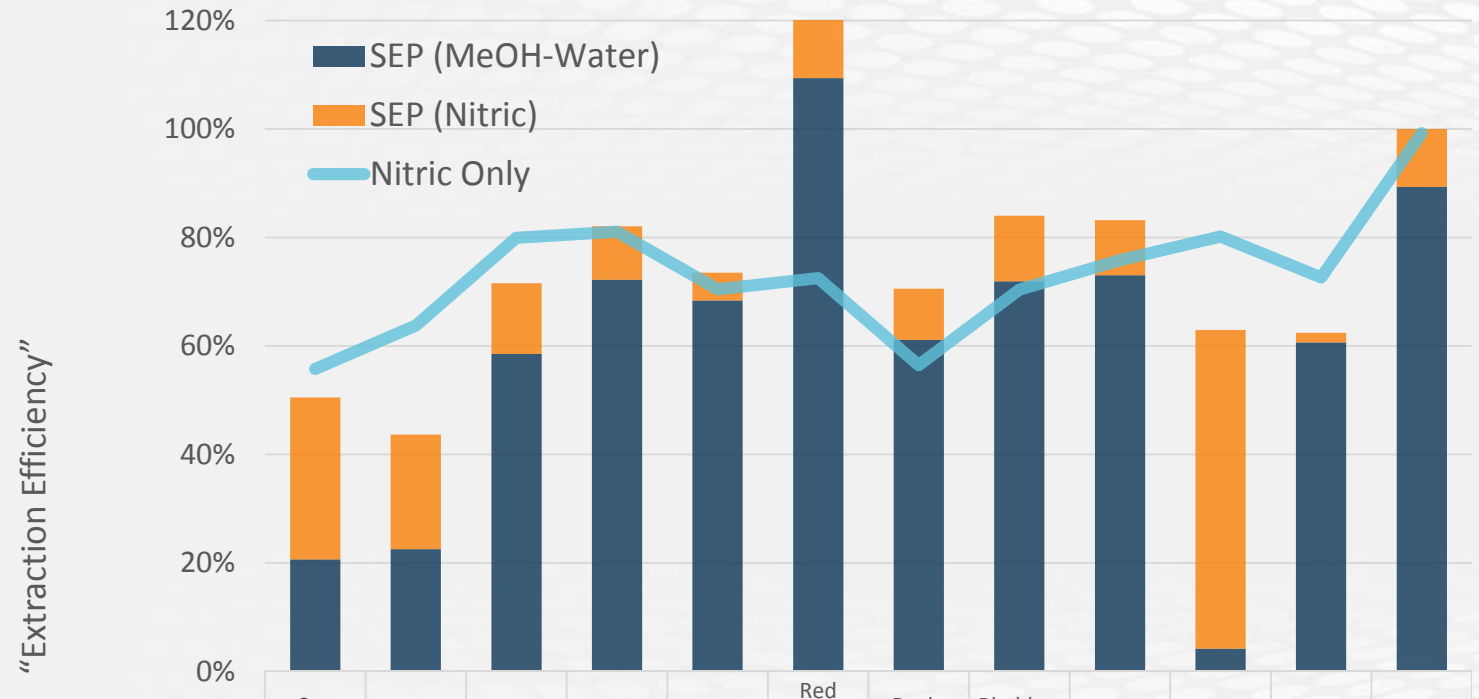
All results in µg/g (ppm)

- LOQs:
  - Hydride ≃ 0.025 µg/g
  - Nitric ≃ 0.020 µg/g
  - SEP ≃ 0.040 µg/g
- Matrix spike recoveries for arsenite and arsenate for all methods ranged from 82 – 115% ($\bar{x}$ = 100%, s = 11%)
- Are differences due to false positives or extractability issues?

Inorganic As Comparison



“Extraction Efficiencies” - Sequential Extraction Procedure vs. Nitric Only



	Sea Lettuce	Aonori	Nori	Laver	Dulse	Red Marine Algae	Rock-weed	Bladder-wrack	Alaria	Wakame	Kombu	Hijiki
SEP (Nitric)	30%	21%	13%	10%	5%	11%	9%	12%	10%	59%	2%	11%
SEP (MeOH-Water)	21%	23%	59%	72%	68%	109%	61%	72%	73%	4%	61%	89%
Nitric Only	56%	64%	80%	81%	70%	73%	56%	70%	76%	80%	73%	99%

- Due to time constraints, “**extraction efficiency**” for this study was calculated using the sum of peaks detectable by IC-ICP-CRC-MS
- Except for the Hijiki and Red Marine Algae samples, “extraction efficiencies” were not quantitative

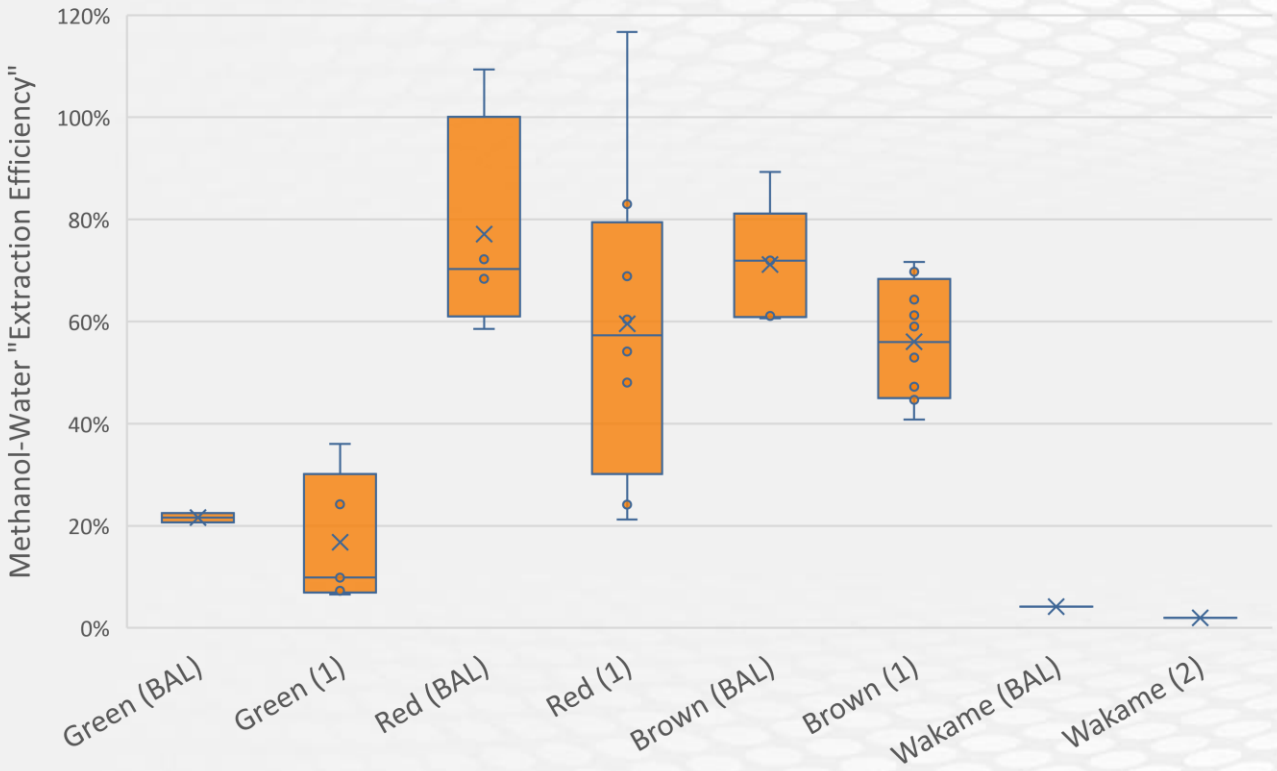
“Extraction Efficiency” of EPA 1632

- Total As data on the extracts pending…
- For two samples which yielded higher inorganic As results via the hydride method, analyzed 2M HCl extracts via IC-ICP-MS

Type	Sample Name	Total As	Hydride Inorg. As	IC-ICP-MS of Hydride (2M HCl) Extracts	Hydride "Extraction Efficiency"	Nitric Only Inorg. As	Nitric Only "Extraction Efficiency"	SEP Inorg. As	SEP "Extraction Efficiency"
Green Algae	Sea Lettuce	8.33	0.183	0.194	83%	0.100	56%	0.085	50%
Brown Algae	Rockweed	27.5	0.564	0.594	46%	0.417	56%	0.373	71%

- Data suggests extractability *might* be the issue

Methanol-Water "Extraction Efficiency" by Algae Type



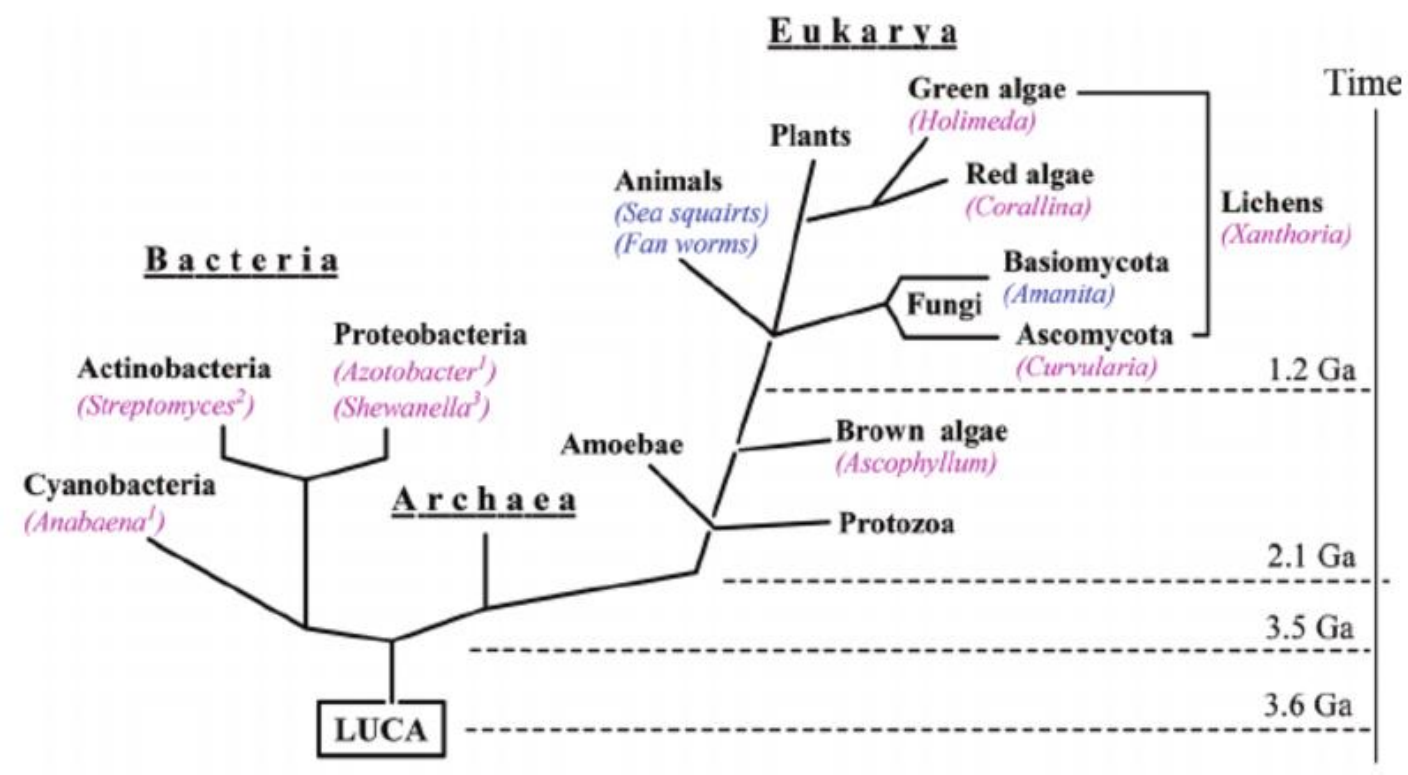
- Total As concentrations among algae are known to vary – generally brown > red or green
- Is extraction efficiency also impacted by algae taxonomy?
- Much more data is needed…

BAL = Brooks Applied Labs (This Study)

1 = V. Taylor and B. Jackson, *Concentrations and speciation of arsenic in New England seaweed species harvested for food and agriculture*, In Chemosphere, Volume 163, 2016, Pages 6-13.

2 = V. Taylor, et. al., *Distinct arsenic metabolites following seaweed consumption in humans*, In Scientific Reports 7, 3920 (2017).

Phylogenetic Tree for Algae



Source: *Dalton Trans.*, 2013,42, 11749-11761

Conclusions

- Analytical methods **must** be optimized for the matrix of interest
  - Test by performing blanks spikes, matrix spikes, and post (analytical) spikes with every As species possible to identify biases or conversion due to the extraction method, the sample matrix, or the analytical method.
  - Include an SRM with every batch
    - NIST 3232 (Kelp Powder) very recently available with reference values for arsenosugars and arsenate (for MeOH-water extraction)
  - A key advantage of LC-ICP-MS is the ability to monitor non-target species (not all species hydride-active)

- This study again confirms that a MeOH-water extraction is better at preserving As species in algae than nitric acid-based extractions, but may not quantitatively extract As
  - Could arsenolipids be part of the missing As?

Conclusions (continued) and Additional Work

- A sequential extraction-based approach can be useful for providing more complete speciation information
  - Since recent studies indicate that arsenosugars are metabolized (unlike arsenobetaine) and can produce cytotoxic intermediates[1], a sequential extraction approach is superior to those that induce species conversion
- Data suggests that extraction efficiencies may be influenced by algae taxonomy
  - If true, this has significant implications for intercomparison studies and CRMs; need diverse range of algae samples to ensure robustness of a method!
  - Hijiki is not the most challenging material for intercomparisons or CRMs
- Additional work to be done:
  - Determine actual extraction efficiencies (not just chromatographic recoveries)
  - Further investigate HCl extraction followed by IC-ICP-MS analysis
  - Further optimize the sequential extraction approach for algae As speciation

[1] Leffers, L., Ebert, F., Taleshi, M. S., Francesconi, K. A. and Schwerdtle, T. (2013), *In vitro toxicological characterization of two arsenosugars and their metabolites*. Mol. Nutr. Food Res., 57: 1270–1282.

Thanks to all Brooks Applied Labs Staff!

