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Transformation of arsenic lipids in decomposing *Ecklonia radiata*

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Abstract

To investigate the release and degradation of arsenolipids present in the marine brown macroalga *Ecklonia radiata*, tissues were collected in various stages of decomposition from intertidal environments, while tissues were also decomposed in laboratory-based microcosms prepared using combinations of autoclaved and natural (non-autoclaved) seawater and sand. Field collected macroalgae samples contained 20–120 μg g^{-1} total As of which 1–10% were arsenolipids comprising mainly an arsenic hydrocarbon (AsHC; 3–13% of total arsenolipids) and four di-acyl arsenic phospholipids (AsPLs; 86–95%). Additionally, a mono-acyl AsPL was found in all water-column decomposing samples. Arsenolipid concentrations in live tissues were similar to those in tissues decomposing in the water-column (1.3–2.9 μg g^{-1} dry mass), which were both up to four times higher than those in decomposing tissues collected from intertidal environments (0.7–1.3 μg g^{-1} dry mass). In the microcosm experiments, the arsenolipid content of *E. radiata* decreased substantially as decomposition proceeded. In the majority of microcosms, more than 75% of the arsenolipids present initially disappeared within 5 days with only the AsHC persisting until day 60 (the length of the experiment). This study demonstrates that the habitat in which decomposition occurs influences the release and degradation of arsenolipids with the greatest losses occurring when tissues decompose in intertidal environments. Microbial diversity, biomass, and overall activity are thus likely to play important roles in the persistence of arsenolipids in decomposing algae.

Keywords Arsenolipids · Biodegradation · Macroalgae · Microcosms · HPLC-ICPMS/ESMS

Introduction

Marine organisms naturally contain high concentrations of organic arsenic species (Edmonds and Francesconi 2003). Currently, more than 100 arsenic species have been identified with arsenoribosides the most common species in algae and arsenobetaine (AB), the dominant species in most marine animals (Francesconi and Edmonds 1998; Rahman et al. 2012).

Many studies have investigated the cycling and biosynthesis of water-soluble As species in marine ecosystems (e.g., Santosa et al. 1994; Rahman et al. 2012; Hanaoka and Usui 2014; Maher et al. 2015; Foster and Maher 2016). Despite arsenic-containing lipids (arsenolipids; the major classes are depicted in Fig. S1) originally being identified over 30 years ago by Morita and Shibata (1988), far less information exists on the production and biosynthesis of arsenolipid species by marine organisms (Sele et al. 2012; Amayo et al. 2014; Petursdottir et al. 2015; Glabonjat et al. 2018). In recent years, however, there has been renewed interest in the characterization of arsenolipids in marine organisms; and as a result, over 80 arsenolipids including As-hydrocarbons (AsHCs) (Taleshi et al. 2008), As-fatty acids (Rumpler et al. 2008), As-fatty alcohols (Amayo et al. 2013), As-phospholipids (AsPLs) (Morita and Shibata 1988; Viczek et al. 2016), and methoxy As-sugar phytol (Glabonjat et al. 2017) have been identified from a range of organisms such as marine algae, squid, and fish.

Algae (macro and unicellular) play three important roles in the cycling of As in marine environments. Firstly, they are able to remove arsenic from the water-column and bio-

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transform inorganic As species (predominantly arsenate) to arsenoribosides and other organic As species such as methylarsonic acid (MA) and dimethylarsinic acid (DMA) (Rahman et al. 2012; Duncan et al. 2015). Secondly, as algae are the bases of most marine food webs, this accumulated and bio-transformed As is then transferred to higher marine organisms that utilize algae as a food source (Rahman et al. 2012; Foster and Maher 2016). Thirdly, the arsenic accumulated by algae is eventually returned to the environment when tissues decompose in the water column, or by active excretion of inorganic and methylated arsenic species, which represents an important link in the marine As-cycle (Hanaoka et al. 1995; Duncan et al. 2014a, b).

Previously, our group investigated the degradation of water-soluble As species associated with the marine macro-alga *Ecklonia radiata* when tissue decomposition occurred in the field and in laboratory microcosms (Duncan et al. 2014a, c). The current study complements those previous findings by utilizing the same tissue samples to investigate the fate of arsenolipids associated with decomposing *E. radiata*. Our objective was to determine how arsenolipid species present in *E. radiata* changed depending on the stage of decomposition and habitat (water-column, intertidal sand/rock platforms), and establish the potential roles played by microbial communities during the decomposition processes.

Materials and methods

Live and naturally decomposing *Ecklonia radiata*

Detailed information on the collection of *Ecklonia radiata* tissues can be found in the study of Duncan et al. (2014c). *Ecklonia radiata* samples were collected between 24 and 26 February 2012 at two estuaries, Lake Tabourie (35.4486° S, 150.4011° E), Burill Lake (35.3886° S, 150.4447° E), and Ulladulla Harbor (35.3667° S, 150.4667° E) in southern New South Wales, Australia. These locations were chosen because there are no arsenic contamination sources and all locations contained live *E. radiata* tissues plus *E. radiata* decomposing in the water-column. In addition, these sites also contained a combination of beach (i.e., sand) and rock platform environments, which we hypothesized, would significantly influence the extent of terrestrial decomposition of the algae and thus would result in different rates of degradation of arsenolipids. Five replicates of live *E. radiata* specimens (live; $n = 5$), *E. radiata* tissues decomposing in the water-column (submerged; $n = 5$), and *E. radiata* tissues decomposing on a sand/rock platform on the beach (beach; $n = 5$) were collected at all three sampling locations. For the analysis of arsenolipids, replicates were pooled within each of the three sampling habitats (live, submerged, and beach).

Microbially manipulated microcosms

Details of the preparation of laboratory microcosms can be found in the study of Duncan et al. (2014a). In brief, *E. radiata* tissue, sand, and seawater were collected from one site at Bawley Point, New South Wales (35.5199° S; 150.3967° E). On return to the laboratory, three subsamples each of *E. radiata*, seawater, and sand were frozen for later use in the microcosm experiments.

Microcosms were created using 225 cm^2 non-pyrogenic tissue culture flasks (Corning, USA) containing *E. radiata* tissue (2 g wet mass), sand (300 g dry mass, either autoclaved or natural), and seawater (600 mL, either autoclaved or natural), which were added to each flask under aseptic conditions. Four sets of conditions, each done in triplicate, were used to establish the microcosms: (1) natural sand and seawater, (2) natural sand and sterile seawater, (3) sterile sand and natural seawater, and (4) sterile sand and sterile seawater. Microcosms were incubated at a light intensity of 110 μmol photons m^{-1} s^{-1}, under a 12:12 h light:dark ratio. Temperatures were maintained at 20 °C during dark periods ramping up to 25 °C during light periods. After incubation for 5 days, one set of samples ($n = 3$ replicates) for each of the experimental treatments ($n = 4$) ($n = 12$ microcosms in total) were randomly selected and removed from the chamber. This process was repeated at all time intervals (days 5, 10, 15, 20, 30, 45, and 60). *E. radiata* tissues were removed and placed into 50-mL centrifuge tubes (polypropylene; Greiner, USA), frozen and freeze-dried for later determination of total arsenic and arsenic species. For the analysis of arsenolipids, replicates from each sampling time were pooled; at some time intervals (natural sand and seawater: days 10, 15, and 30; sterile seawater: days 20, 30, and 60; and sterile sand: day 15), there was insufficient algal biomass for arsenolipid measurements.

Reagents and standards

Water used for all analytical steps was obtained from a Milli-Q system (18.2 MΩ cm, Millipore GmbH, Austria). Methanol (≥ 99.9%, MeOH), dichloromethane (≥ 99.9%, DCM), formic acid (≥ 98%), acetone (≥ 99.5%), and ammonia (25%) were obtained from Carl Roth GmbH (Germany); acetone (≥ 99.5%) was purchased from Sigma-Aldrich (Austria); and acetic acid (100% glacial) and silica gel 60 (0.063–0.200 mm) were obtained from Merck (Switzerland). The certified reference material was NMIJ CRM 7405-a (Trace Elements and Arsenic Compounds in Seaweed – Hijiki) from the Natural Metrology Institute of Japan (Tsukuba, Ibaraki, Japan).

Standard arsenolipids, namely AsHC332, AsHC360, and AsHC444 (structures are shown in Fig. S1), were synthesized in-house according to Taleshi et al. (2014), and standard solutions (each 0.1 mM or 7.5 mg L^{-1}) were prepared by dissolving the compound (7.5 ± 0.2 μg as As) in MeOH (1 mL).

Arsenolipid measurements

The lipid arsenic species within *E. radiata* tissues were measured via high performance liquid chromatography (HPLC) coupled simultaneously to an inductively coupled plasma mass spectrometer (ICPMS) and an electrospray ionization mass spectrometer (ESMS) (Yu et al. 2018). The freeze-dried algal sample (10 mg weighed to a precision of 0.01 mg) was extracted with DCM/MeOH (2 + 1, *v*/*v*; 1 mL) for 1 h on a rotary cross at room temperature. The mixture was centrifuged (10 min, 5000×*g*), and a portion of the liquid phase (0.7 mL) was filtered (0.2 μm; nylon) and injected onto the HPLC-system.

Separation of arsenolipid species was carried out by reversed-phase (RP) HPLC using an ACE UltraCore SuperC18 column (4.6 × 250 mm, 5-μm particle size). Elution was performed with water containing 25 mM ammonium acetate (adjusted to pH 9.2 with NH_3; mobile phase A) and MeOH containing 25 mM ammonium acetate (adjusted to pH 9.2 with NH_3; mobile phase B) under gradient elution conditions: 0–40 min, 20–100% B; 40–53 min, 100% B; 53–53.5 min, 100–20% B; and 53.5–60 min, 20% B. Flow rate was 1 mL min^{-1}, column temperature was 40 °C, and injection volume was 50 μL. For ICPMS detection, the HPLC effluent was split whereby 10% were transported to the ICPMS (to maintain a stable plasma by reducing the organic solvent load) using a passive splitter (Analytical Scientific Instruments, Richmond, USA) and post-splitter support of the outflow with water containing 1%v/v formic acid and internal standards Ge, In, and Te (20 μg L^{-1}; 0.9 mL min^{-1}) through a T-piece. Carbon compensation (Raber et al. 2010) was performed by constant introduction of an aqueous acetone solution (5%v/v in water) directly into the spray chamber delivered with a rotatory pump (0.2 mL min^{-1}) to ensure constant carbon content reaching the plasma. Limit of detection (LOD) and limit of quantification (LOQ) of the HPLC-ICPMS method were 0.01 μg As g^{-1} dry mass (based on three blank extracts + 3 × standard deviation of the blank) and 0.05 μg As g^{-1} dry mass (based on three blank extracts + 10 × standard deviation of the blank), respectively. Lipid extracts were measured on an Agilent 7900 instrument. For ESMS detection, the remaining 90% of the HPLC effluent was directly introduced to the ion source of an Agilent 6460 instrument equipped with a Jetstream ion source operated in positive ionization mode using a capillary voltage of 5 kV and nozzle voltage of 2 kV; N_2 gas temperature was 350 °C with a flow of 12 L min^{-1}; nebulizer pressure was 25 psi; and sheath gas temperature was 350 °C with a flow of 11 L min^{-1}. Arsenolipid species were extracted from either SCAN mode observing a mass range of m/z 150–1100 with a scan time of 0.5 s, and fragmentor voltages of 135 V and 200 V; or determined in MRM mode with dwell times of 0.1 s per transition using the conditions described in Table S1.

In addition to the simultaneous determination by RP-HPLC-ICPMS/ESMS, we also used high-resolution (HR) ESMS for the accurate measurement of arsenolipid masses using a Thermo Q-Exactive Hybrid Quadrupole Orbitrap MS equipped with an HESI-II ion source (Thermo Scientific, USA). The HPLC system comprised an UltiMate 3000 system (Thermo Scientific). Freeze-dried *E. radiata* samples (100 mg) were extracted with DCM/MeOH (2 + 1, *v*/*v*; 3 mL) for 1 h on a rotary cross at room temperature. The mixture was centrifuged (10 min, 5000×*g*) and a portion of the liquid phase (2.7 mL) evaporated to dryness (100 mbar, 25 °C), re-dissolved in MeOH/acetone (1 + 1, *v*/*v*, containing 1%v/v formic acid, 1 mL), and transferred to a small column containing silica gel 60 (glass Pasteur pipette, 230 × 5 mm, filled to a height of 40 mm), conditioned with the same mixture (5 mL). The column was washed with the conditioning solution (5 mL) and then with pure MeOH (2 mL). Finally, arsenolipids were eluted with MeOH containing 1%v/w NH_3 (8 mL) and evaporated to dryness (10 mbar, 25 °C), and the residue stored at − 20 °C. The residue was re-dissolved in pure MeOH (200 μL) prior to measurement.

Chromatographic conditions were slightly different for HR-ESMS measurements owing to the need for higher sensitivity: HPLC-column was an Asahipak ODP-50 (150 × 4.6 mm; 5 μm particle size); separation of arsenolipids was carried out using gradient elution conditions with a flow of 0.5 mL min^{-1} using water containing 0.1%v/v formic acid (mobile phase A) and MeOH containing 0.1%v/v formic acid (mobile phase B), 0–30 min, 60–100% B; 30–40 min, 100% B; 40–40.1 min, 100–60% B; and 40.1–50 min, 60% B. The HPLC column was held at 40 °C and the injection volume was 20 μL. The HR-ESMS was operated under positive ionization conditions (3.5 kV) in data dependent MS^2 mode fragmenting the five most abundant peaks of each scan event (m/z 100–1200) with fragmentation energies of 20, 35, and 50 instrument units (IU). Resolution was set to 70,000 (FWHM) with an isolation window of 0.4 m/z at the quadrupole; capillary temperature, 270 °C; sheath gas flow, 52.5 IU; auxiliary gas flow, 14 IU; and probe temperature 438 °C.

Data evaluation of ICPMS measurements was carried out with the chromatographic software MassHunter B.01.01 obtained from Agilent Technologies (Germany). Quantification was based on peak areas with external calibration against standards of AsHC332, AsHC360, and AsHC444 for lipid-soluble arsenicals. ESMS data were evaluated with MassHunter Workstation B.07.00 from Agilent Technologies, and HR-ESMS data were evaluated with chromatographic software Xcalibur 3.0.63 from Thermo Scientific (USA). Accuracy of the arsenic speciation procedure was determined by analysis of the certified reference material NMIJ 7405-a Hijiki prepared in the same way as *E. radiata* samples (Fig. S2 and Table S2). Identification of individual arsenolipids was based on retention time matching with CRM Hijiki (RP-HPLC-ICPMS/ESMS), as well as accurate mass determination with RP-HPLC-HR-ESMS (Table S3). Arsenolipid species found in *E. radiata* samples in this study are shown in Fig. 1.

Total arsenic

Total arsenic concentrations in naturally decomposing *E. radiata* tissues and in *E. radiata* decomposing in microbially manipulated microcosms were determined in our previous studies (Duncan et al. 2014a, c).

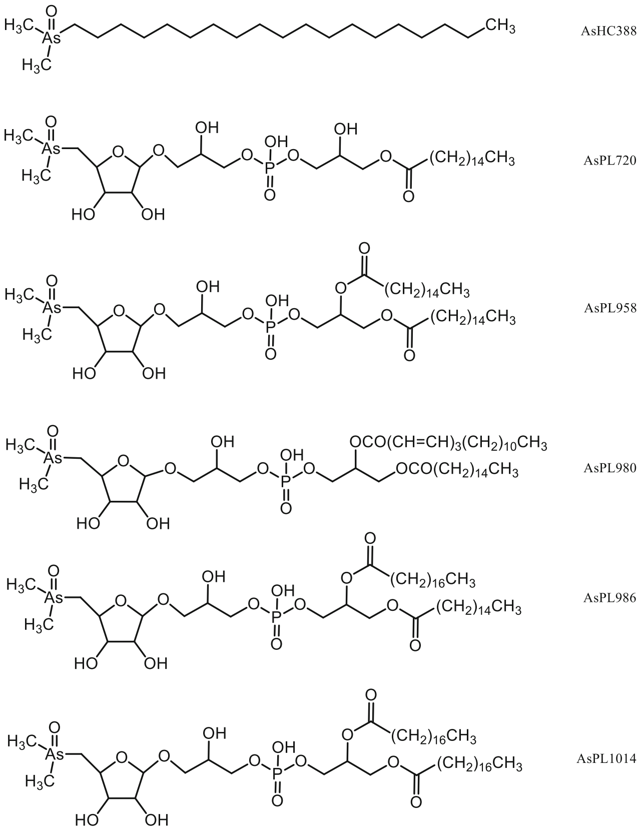


Fig. 1 Arsenic containing lipids found in *Ecklonia radiata* extracts. For unsaturated AsPL980, we show only one possible isomer of the lipophilic side-chains

Results and discussion

Arsenolipids present in live and naturally decomposing Ecklonia radiata tissues

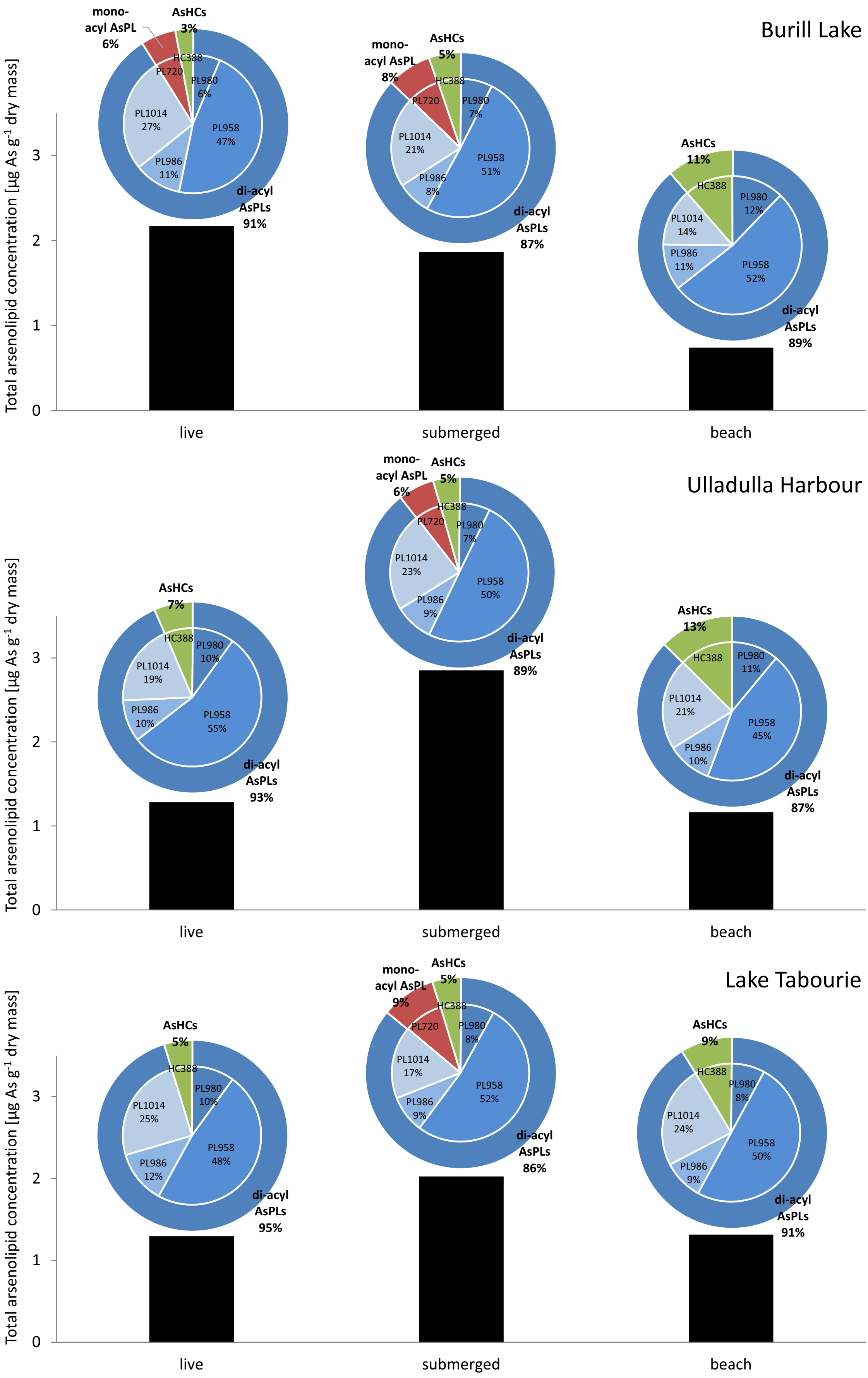
Total arsenolipid concentrations

For live *E. radiata* samples, total arsenolipid concentrations ranged between 1.28 and 2.17 μg g^{-1} dry mass (Fig. 2 and Table S4) across the three sampling locations, which represents ca 1–3% of the total As in the macroalga. This arsenolipid concentration range is consistent with that reported for other marine algae (García-Salgado et al. 2012; Raab et al. 2013; Glabonjat et al. 2014; Al Amin et al. 2018).

Total arsenolipid concentrations in decomposing *E. radiata* tissues collected in the water-column (submerged) ranged between 1.87 and 2.85 μg g^{-1} dry mass across the three sampling locations (Fig. 2 and Table S4). These values were comparable with, or even higher than, those found in the live algae, indicating that the arsenolipids are retained, at least initially, in the submerged *E. radiata* tissues. This is in contrast to the behavior of total arsenic, mostly consisting of water soluble As species, which decreased by 25–65% as a result of abiotic processes such as leaching occurring in the water column (Duncan et al. 2014c). As a consequence, the proportion of arsenic present as arsenolipids in the submerged algae (2–10%) was two- to threefold higher than that found in the live algae.

Total arsenolipid concentrations in decomposing *E. radiata* tissue collected from intertidal environments (beach) ranged between 0.74 and 1.31 μg g^{-1} dry mass (4–6% of total As) with similar concentrations present across all three locations (Fig. 2 and Table S4). These arsenolipid concentrations in algae collected from beach environments were lower than those in live and submerged *E. radiata*, which indicates that the arsenolipids were starting to degrade. The arsenolipids, however, were much more resistant to degradation than were the water-soluble arsenicals investigated in our previous study (Duncan et al. 2014c).

Fig. 2 Total lipid-soluble arsenic concentrations and distribution of individual arsenolipids at three stages of naturally degrading *Ecklonia radiata* collected at three locations. Concentrations were determined by RP-HPLC-ICPMS (*n* = 1) ▶


Burill Lake
Total arsenolipid concentration [µg As g^{-1} dry mass]
0
1
2
3
live
submerged
beach
mono-acyl AsPL 6%
AsHCs 3%
HC388
PL720
PL980 6%
PL1014 27%
PL958 47%
PL986 11%
di-acyl AsPLs 91%
mono-acyl AsPL 8%
AsHCs 5%
HC388
PL720
PL980 7%
PL1014 21%
PL958 51%
PL986 8%
di-acyl AsPLs 87%
AsHCs 11%
HC388
PL980 12%
PL1014 14%
PL986 11%
PL958 52%
di-acyl AsPLs 89%
Ulladulla Harbour
Total arsenolipid concentration [µg As g^{-1} dry mass]
0
1
2
3
live
submerged
beach
AsHCs 7%
HC388
PL980 10%
PL1014 19%
PL986 10%
PL958 55%
di-acyl AsPLs 93%
mono-acyl AsPL 6%
AsHCs 5%
HC388
PL720
PL980 7%
PL1014 23%
PL958 50%
PL986 9%
di-acyl AsPLs 89%
AsHCs 13%
HC388
PL980 11%
PL1014 21%
PL958 45%
PL986 10%
di-acyl AsPLs 87%
Lake Tabourie
Total arsenolipid concentration [µg As g^{-1} dry mass]
0
1
2
3
live
submerged
beach
AsHCs 5%
HC388
PL980 10%
PL1014 25%
PL958 48%
PL986 12%
di-acyl AsPLs 95%
mono-acyl AsPL 9%
AsHCs 5%
HC388
PL720
PL980 8%
PL1014 17%
PL958 52%
PL986 9%
di-acyl AsPLs 86%
AsHCs 9%
HC388
PL980 8%
PL1014 24%
PL986 9%
PL958 50%
di-acyl AsPLs 91%

Release and degradation of arsenolipid species

All the *E. radiata* samples contained AsHC388, constituting 3–13% of total arsenolipids, and four di-acyl AsPLs, which together made up 86–95% of the total arsenolipids, while mono-acyl AsPL720 (6–9%) was mainly found in samples decomposing in the water column (submerged) (Fig. 2 and Table S4). The proportions of arsenolipid species in live *E. radiata* were similar across locations with AsPL958 the dominant species (48–55%) followed by AsPL1014 (19–27%), AsPL986 (10–13%), AsPL980 (6–11%), and AsHC388 (3–6%). All the arsenolipids found here have been reported previously in a range of macroalgal species (García-Salgado et al. 2012; Raab et al. 2013; Yu et al. 2018) albeit in different proportions. Most studies have observed AsPLs to be abundant in macroalgae with lesser amounts of AsHCs (García-Salgado et al. 2012; Al Amin et al. 2018), although Petursdottir et al. (2015) observed the opposite pattern in *Ectocarpus* spp.

The proportions and concentrations of arsenolipids in decomposing *E. radiata* tissues collected in the water column (submerged) were similar to those found in live *E. radiata* tissues (Fig. 2, Fig. S2 and Table S4). There was, however, one exception: AsPL720 was present in all *E. radiata* tissues decomposing within the water-column, whereas this arsenolipid was only detected in one live algal sample (Fig. 2 and Table S4). Unlike the other AsPLs detected here, AsPL720 is a mono-acylated species and therefore it is likely to arise as a first stage degradation product of the di-acylated As-phospholipids. Mono-acyl arsenolipids have only rarely been reported in algae (Yu et al. 2018).

In extensively decomposed (beach) *E. radiata* tissue samples, the AsPLs remained in similar proportions to those in live and submerged tissues (Fig. 2 and Table S4). This pattern was maintained even in the Burill Lake samples where the total arsenolipid concentrations in beach algae were markedly lower than those in the live samples. In contrast, the proportion of AsHC388 increased more than twofold in the beach decomposing samples (live 3–6%, submerged 4–6%, beach 9–13%) indicating that the AsHCs were more resistant to degradation than were the AsPLs. But overall, the arsenolipids were relatively stable in decomposing algal tissue, and thus their fate was markedly different from the fate of the water-soluble arsenic species, mainly arsenoribosides, which readily degraded under the same conditions (Duncan et al. 2014c).

Arsenolipids in Ecklonia radiata decomposing in microbially manipulated microcosms

Total arsenolipid concentrations

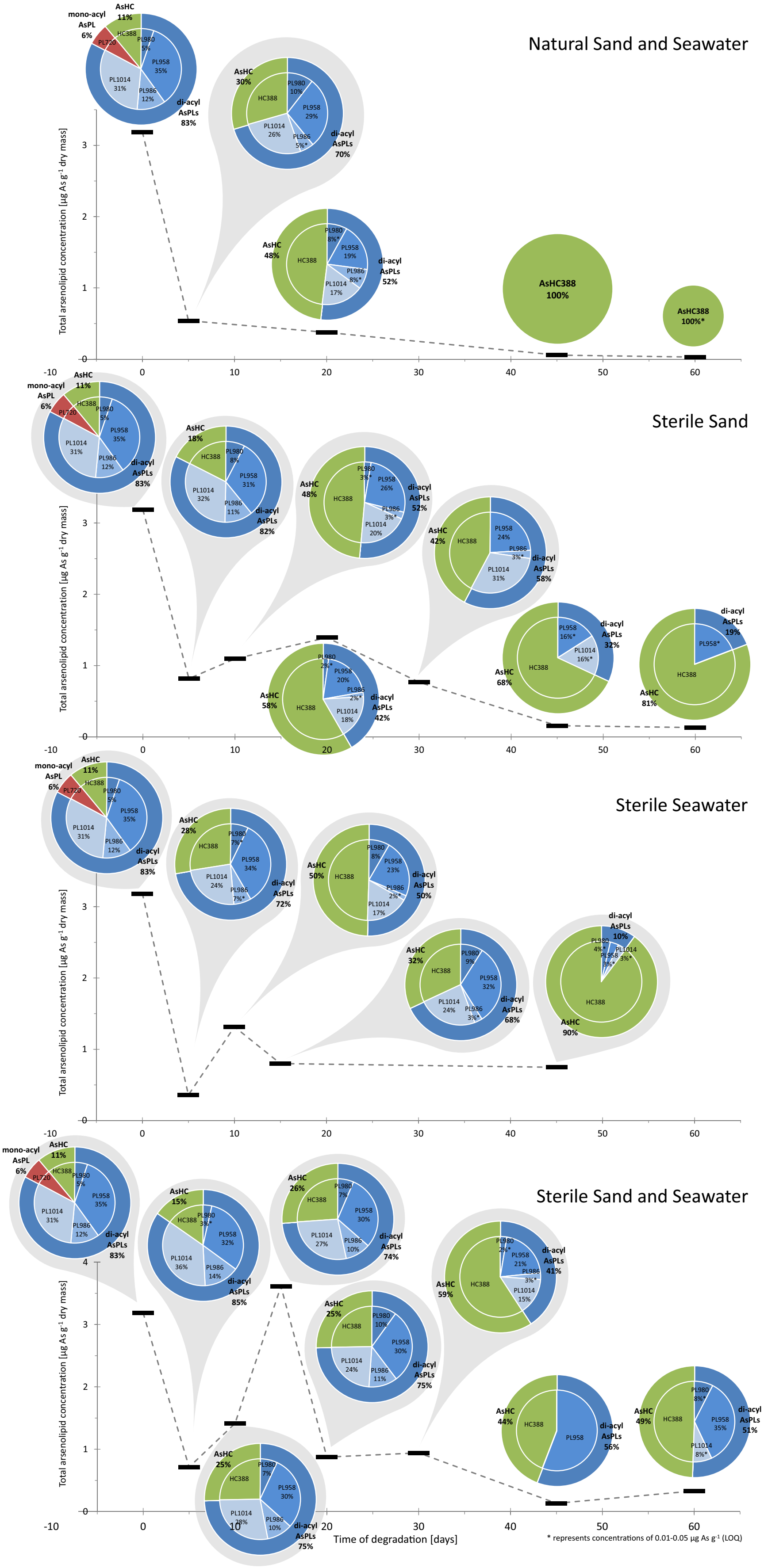
Initial total arsenolipid concentrations in the field collected *E. radiata* tissue was ca 3 μg As g^{-1} dry mass. The total lipid-soluble As in all microcosms decreased by more than 75% during the first 5 days of incubation, and by the conclusion of the experiments (day 60), less than 10% of the initially present arsenolipids was present in the decomposing *E. radiata* tissues (Fig. 3 and Table S5). These rates of degradation were much faster than those observed for the field-based experiments, presumably reflecting increased microbial activity in the microcosms. We observed the fastest and most constant release of arsenolipids from *E. radiata* tissues under conditions with natural sand and seawater (Fig. 3 and Table S5).

Release and degradation of arsenolipid species

In live *E. radiata***,** the proportions of arsenolipids were AsHC (11%; 0.35 μg As g^{-1} dry mass), mono-acyl AsPL (6%; 0.20 μg As g^{-1})**,** and di-acyl AsPLs (83%; 2.63 μg As g^{-1}) with the major components being AsPL958, AsPL980, As PL986, AsPL1014, AsHC388, and AsPL720 (Fig. 3, Fig. S2 and Table S5). In microcosms with natural sand and seawater**,** the concentrations of AsHC and di-acyl AsPLs reduced by 55 and 85%, respectively after the first 5 days; while the mono-acyl AsPL720 was not detected in any decomposing tissue sample (Fig. 3 and Table S5). This rapid loss of arsenolipids was similar to that found for water-soluble arsenicals in decomposing algae as reported in our previous study (Duncan et al. 2014a) and by others (Edmonds et al. 1982; Pengprecha et al. 2005; Navratilova et al. 2011; Ojo and Onasanya 2013). By the latter stages of the experiment (days 45–60), almost all the AsPLs had degraded and only AsHC388 was detected in measurable concentrations (Fig. 3 and Table S5). This finding is consistent with the data described earlier from the field collected E. radiata, which showed that the AsHCs were more resistant to degradation than the AsPLs.

In the three microcosms using sterile sand, sterile seawater, or a combination of both, the degradation of the arsenolipids was similar to that observed for the natural sand and seawater microcosm, albeit less extensive. The AsHCs were again more recalcitrant than the AsPLs; by the conclusion of the experiments, more than 90% of all AsPLs had degraded whereas up to 50% of the AsHCs remained associated with the *E. radiata* tissues (Fig. 3 and Table S5). The similarity in arsenolipid degradation across the various treatments (natural sand and seawater versus sterile sand/seawater combinations) suggests that microbes associated with the surfaces of *E. radiata* may have re-colonized the initially sterile seawater and sand

Fig. 3 Total lipid-soluble arsenic concentrations and distribution of individual arsenolipids in *Ecklonia radiata* at various stages of degradation in microcosms under four sets of conditions. Concentrations were determined by RP-HPLC-ICPMS (*n* = 1) ▶


Natural Sand and Seawater
Sterile Sand
Sterile Seawater
Sterile Sand and Seawater
Total arsenolipid concentration [µg As g⁻¹ dry mass]
Time of degradation [days]
-10
0
10
20
30
40
50
60
0
1
2
3
4
AsHC 11%
mono-acyl AsPL 6%
di-acyl AsPLs 83%
HC388
PL720
PL980 5%
PL958 35%
PL986 12%
PL1014 31%
AsHC 30%
PL980 10%
PL958 29%
PL986 5%*
PL1014 26%
di-acyl AsPLs 70%
AsHC 48%
PL980 8%*
PL958 19%
PL986 8%*
PL1014 17%
di-acyl AsPLs 52%
AsHC388 100%
AsHC388 100%*
AsHC 18%
PL980 8%
PL958 31%
PL986 11%
PL1014 32%
di-acyl AsPLs 82%
AsHC 48%
PL980 3%*
PL958 26%
PL986 3%*
PL1014 20%
di-acyl AsPLs 52%
AsHC 42%
PL958 24%
PL986 3%*
PL1014 31%
di-acyl AsPLs 58%
AsHC 58%
PL980 2%*
PL958 20%
PL986 2%*
PL1014 18%
di-acyl AsPLs 42%
AsHC 68%
PL958 16%*
PL1014 16%*
di-acyl AsPLs 32%
AsHC 81%
PL958*
di-acyl AsPLs 19%
AsHC 28%
PL980 7%*
PL958 34%
PL986 7%*
PL1014 24%
di-acyl AsPLs 72%
AsHC 50%
PL980 8%
PL958 23%
PL986 2%*
PL1014 17%
di-acyl AsPLs 50%
AsHC 32%
PL980 9%
PL958 32%
PL986 3%*
PL1014 24%
di-acyl AsPLs 68%
AsHC 90%
PL980 4%*
PL958 3%*
PL1014 3%*
di-acyl AsPLs 10%
AsHC 15%
PL980 3%*
PL958 32%
PL986 14%
PL1014 36%
di-acyl AsPLs 85%
AsHC 25%
PL980 7%
PL958 30%
PL986 10%
PL1014 28%
di-acyl AsPLs 75%
AsHC 26%
PL980 7%
PL958 30%
PL986 10%
PL1014 27%
di-acyl AsPLs 74%
AsHC 25%
PL980 10%
PL958 30%
PL986 11%
PL1014 24%
di-acyl AsPLs 75%
AsHC 59%
PL980 2%*
PL958 21%
PL986 3%*
PL1014 15%
di-acyl AsPLs 41%
AsHC 44%
PL958
di-acyl AsPLs 56%
AsHC 49%
PL980 8%*
PL958 35%
PL1014 8%*
di-acyl AsPLs 51%
* represents concentrations of 0.01-0.05 µg As g⁻¹ (LOQ)

environments. Thus, by day 60, differences in the overall microbial biomass and activity may have been minimal. Alternatively, the decomposition of *E. radiata* may have been autocatalytic or abiotic in nature (Kolter and Sandhoff 2010; Rontani et al. 2014).

This study is the first to document the arsenolipid species in decomposing marine macroalgae and track the degradation of arsenolipid species over time. Overall, the results show that most of the AsPLs species (mono- and di-acylated) are rapidly lost from tissue during the early stages of decomposition especially when detached algae are allowed to settle on the ocean floor. As decomposition proceeds (i.e., within intertidal zones or on the ocean floor), AsPLs are further degraded; and over time, they are lost completely from algal tissues. AsHC388, however, appears to be more persistent although reasons for this are currently uncertain. Further research is thus required to fully understand and characterize the arsenolipid degradation pathways that occur in marine environments.

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