Vol. 15: 215–224, 2012
doi: 10.3354/ab00431

AQUATIC BIOLOGY
Aquat Biol

Published June 6

Chemical and physical features of living and non-living maerl rhodoliths

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ABSTRACT: Living (LM) and non-living maerl (NLM) rhodoliths of the species *Lithothamnion corallioides* (Crouan & Crouan, 1867) from Kingstown Bay, Galway, were sampled and compared in relation to their physical structure and lipid and low molecular weight carbohydrate (LMWC) composition. Saturated (SATFA) and polyunsaturated fatty acids (PUFA) were dominant, in particular 16:0, 20:4n-6 and 20:5n-3, but a diverse range of fatty acids were identified. The abundance of *n*-alkanals was high, and sterol composition was simple, with cholesterol accounting for over 90% of the total sterols. Mono-, di-, and trisaccharides, with galactose units being dominant, and floridoside were present in high abundance. Notably, the fatty acid and LMWC profiles varied little between NLM and LM. The relatively high abundance of PUFA and floridoside, in particular, suggests that NLM may have further potential for research and commercial purposes in a variety of food, biomedical and industrial applications. Previously reported unidentified 'globular inclusions' were more abundant in NLM and exhibited a crystalline morphology. Together with the bacterial fatty acid composition of LM and NLM, the results indicate that these structures are not bacterial in nature.

KEY WORDS: Maerl · Coralline algae · Fatty acids · Floridoside · Lipids · Kingstown Bay · Galway

INTRODUCTION

Maerl is the collective name for a group of non-geniculate calcareous red algae (rhodoliths) from the family *Corallinaceae*, of which *Phymatolithon calcareum* (Pallas) (Adey & McKibben 1970) and *Lithothamnion corallioides* (Crouan & Crouan 1867) are the 2 primary maerl bed-forming species (Blunden et al. 1997, Foster 2001). Maerl beds occur globally (Bosence 1983, Freiwald & Henrich 1994) and may be one of the world's 4 major marine benthic communities, along with kelp, seagrasses and non-geniculate corals (Foster 2001). Maerl beds are now known to function as spatially complex but sensitive habitats that support a rich and fragile biodiversity (Barbera et al. 2003, Blake & Maggs 2003, Bosence & Wilson 2003). The recent BIOMAERL project has shown that macroalgae, annelids, crustaceans and molluscs are particularly diverse in many maerl habitats (BIOMAERL Team 1999).

Living maerl (LM) is reddish-purple in colour and possesses abundant epiflora, while non-living maerl (NLM) is typically greyish-white to brown-white and lacks epiflora (Blunden et al. 1975). It is primarily NLM that is commercially exploited (Blunden et al. 1975) and has been collected off the Irish, Cornish and Brittany coasts since the 17th, 18th and 19th centuries respectively (De Grave & Whitaker 1999). It is used as an agricultural fertiliser to increase the pH of acidic soils and also more recently for soil ameliora-

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tion, as an animal fodder additive, in acid water treatment, in biological denitrification, in drinking water treatment, in toxin elimination and in the pharmaceutical, cosmetics, nuclear and medical industries (Blunden et al. 1975, 1977, 1997).

The primary negative anthropogenic effects on maerl habitats are smothering with fine sediment from trawling and commercial exploitation (De Grave & Whitaker 1998, Bordehore et al. 2003, Grall & Hall-Spencer 2003, Wilson et al. 2004) and eutrophic conditions due to sewage and fish discharge (Wilson et al. 2004). The European Commission Council Directive 92/43/EEC, commonly known as the 'Habitats Directive', lists *Lithothamnion corallioides* and *Phymatolithon calcareum* in Annex V as being subject to management and conservation (European Council 1992). However, this only applies to Atlantic exploited maerl and not to any rarer species or non-commercially exploited maerl (Barbera et al. 2003). Data regarding the distribution of Irish maerl beds has been the topic of a recent report (De Grave & Whitaker 1999), which concludes that 65 to 70% of all confirmed maerl beds in Irish waters occur in the Galway-Connemara region, 20 to 25% occur in the southwest region, and the remainder occur along the Donegal coast, with an overall abundance of the order of 3×10^6 m^3 of exploitable maerl. With this vast resource, there has been renewed interest in maerl, both commercially and in terms of its ecological importance and conservation needs.

To date, the primary focus of research on maerl has been ecological and distribution studies (Barbera et al. 2003, and references therein). Previous studies have been carried out to characterise its physical and inorganic chemical composition (Borowitzka et al. 1974, Blunden et al. 1975, 1997), but there are few studies building on this work. Furthermore, coralline red algal deposits have been useful in paleoclimatic and paleoenvironmental reconstructions regarding global sea-level fluctuations (Titschack et al. 2008), seasonal climate variability (Freiwald & Henrich 1994) and the use of the deposits as bi-weekly paleothermometers (Kamenos et al. 2008), but there have been no such applications using maerl deposits. To our knowledge, there has been no study of the biochemical composition of maerl and no comparison of the structural and compositional differences between LM and NLM to date. The purpose of the present study is to report on the solvent-extractable lipid and low molecular weight carbohydrate (LMWC) composition of LM and NLM from Kingstown Bay, Galway, Ireland, and to compare the physical and chemical composition of LM and NLM.

MATERIALS AND METHODS

Sampling and sample preparation

Dead and living maerl thalli were sampled using a benthic grab sampler in Kingstown Bay (53° 30.738′ N, 10° 6.486′ W) in 2009 (Fig. 1). Samples were stored at 4°C and a portion also at −20°C prior to analysis. These samples were positively identified (AQUAFACT International Services) as *Lithothamnion corallioides* (Crouan & Crouan, 1867). The samples were meticulously cleaned by physical removal of large flora, fauna and debris and the removal of adhered biomass and organic matter using tweezers and a small brush, followed by washing with deionised water. Sample aliquots were oven-dried at 110°C or lyophilised before grinding using a mortar and pestle and sieving through a 250 µm sieve.

Physical and chemical analysis

Four representative thalli from LM and NLM samples were snapped according to Blunden et al. (1997) and adhered to carbon tabs. The samples were then sputter-coated with ~30 nm Au and imaged at 10 kV accelerating voltage using a HITACHI® S-3000N scanning electron microscope. X-ray diffraction was performed on oven-dried (110°C) powdered samples with a Philips® X'Pert X-ray diffractometer using Cu$K\alpha$ radiation. The samples were measured in step-scan mode with steps of 0.04 (°2θ) and a counting time of 1 s. Infrared (IR) spectroscopy was performed using an Avatar 320 FT-IR spectrophotometer. Maerl samples were ground and mixed with KBr at an opti-

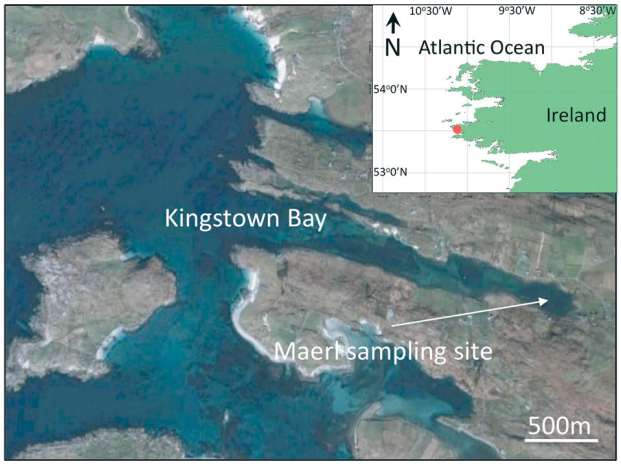

Fig. 1. Maerl sampling site, Kingstown Bay, Galway, Ireland (adapted from INFOMAR data and GOOGLE Earth™)

mum ratio of 1:1000 (1 mg of sample in 1 g of KBr), and a KBr pellet was prepared. Limestone was used as a comparison. Data was collected and processed using the EZ OMNIC (V6) operating software.

Lipid and carbohydrate extraction

All solvents (ROMIL-SpS®), standards and reagents (Sigma Aldrich®) were high-purity GC-grade. All glassware was heated for 8 h at 450°C. Sub-samples of 2.5 g freeze-dried maerl were extracted by sequential ultrasonic-assisted extraction for 30 min each using the following solvent regime: 15 ml CH_3OH, followed by 15 ml 1:1 CH_2Cl_2:CH_3OH and finally 15 ml CH_2Cl_2. Solvent extracts were combined, vacuum-filtered through furnace-treated glass fibre filters (Whatman® GF/A) and reduced by rotary evaporation before being transferred to vials.

Gas chromatography-mass spectrometry

Total extracts were dried fully under N_2 gas and reconstituted in 1:1 (v/v) CH_2Cl_2:CH_3OH. An aliquot was used to convert ester-linked lipids and free fatty acids to fatty acid methyl esters (FAMEs) using 0.5 M sodium methoxide. A second aliquot of the total extract was used to silylate hydroxy-functional groups using 9:1 (v/v) N-O-Bis-(trimethylsilyl) trifluoroacetamide (BSTFA)/pyridine. Aliquots (1 µl) of derivatised extracts were injected in splitless mode onto an Agilent® 6890 N gas chromatograph interfaced with an Agilent® 5975C mass selective detector (MSD). A HP-5MS fused silica capillary column (30 m × 0.25 mm i.d. and film thickness of 0.25 µm, Agilent®) was used with high purity helium carrier gas at a constant 1.3 ml min^{-1} flow rate. The injector and mass spectrometer source were held at 280°C and 230°C respectively. The column temperature program was as follows: 65°C injection and hold for 2 min, ramp at 6°C min^{-1} to 300°C followed by isothermal hold at 300°C for 20 min. The mass spectrometer was operated in electron impact mode with an ionisation energy of 70 eV, with the mass scan range set from 50 to 650 Da.

Data was acquired and processed using Chemstation software (Version G1701EA). The monounsaturation position was identified on FAMEs by formation of DMDS adducts (Nichols et al. 1986), and all compounds were confirmed using a combination of NIST® and Wiley® mass spectral libraries, interpretation of mass fragmentation patterns and compound retention times and comparison with literature (e.g. Fang et al. 2006, Volkman 2006, Medeiros & Simoneit 2007, Brokł et al. 2009). Analytes were quantified using total ion current peak area and converted to mass concentrations using multiple-point internal standard calibration curves. For each case, 100 µg ml^{-1} 5-α-cholestane was used as an internal standard, and the analyte model compounds used were tetradecanoic acid methyl ester, D-(+)-glucose, nonadecane, stigmasterol and hexadecanol. Procedural blanks were run to monitor background interferences.

RESULTS

Bulk physical and chemical composition

X-ray diffractograms obtained from powdered LM (upper trace) and NLM (lower trace) are shown in Fig. 2. The peak *d*-spacing is consistent with calcite, and characteristic peaks for aragonite or dolomite were not apparent (Downs & Hall-Wallace 2003). Fig. 3 shows representative IR spectra obtained from NLM, LM and limestone samples. Comparison with limestone highlights the distinct peaks attributable to carbonate, in particular the very strong peaks observed at 1420 cm^{-1}, 874 cm^{-1} and 718 cm^{-1} resulting from C–O bonds. Peaks arising from organic functional groups are apparent in both LM and NLM but are minor. The peak and inverted peak at 2347 cm^{-1} in NLM and LM respectively is likely due to CO_2 impurities.

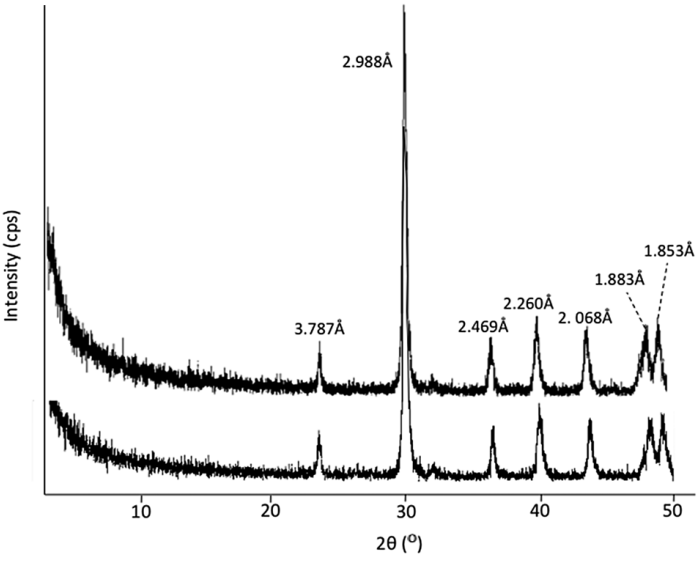

Fig. 2. Living (top) and non-living (bottom) maerl X-ray diffractograms. Peak *d*-spacings are labelled (Å)

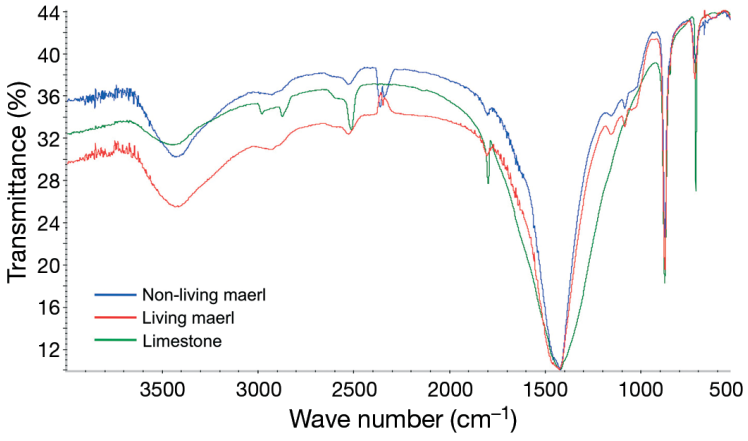

Fig. 3. Infrared spectra from non-living maerl, living maerl and limestone samples

Scanning electron microscopy (SEM)

The calcified structures of NLM and LM thalli were porous and contained globular inclusions (Fig. 4). These structures were significantly more abundant in the NLM than in the LM (Fig. 4). Fig. 5A shows SEM images of abundant globular inclusions (>2 µm) with apparent angular morphology (Fig. 5A, 'X') and also smaller (<1 µm) cocci or streptococci-shaped structures (Fig. 5B, 'Y').

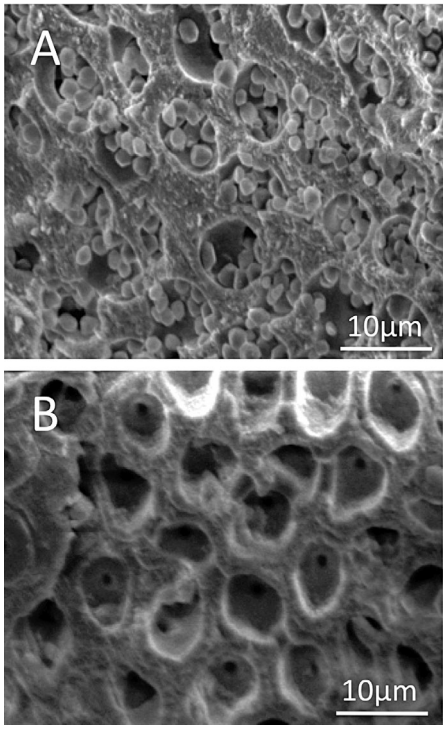

Fig. 4. SEM images of (A) non-living maerl, with abundant globular inclusions, and (B) living maerl, with notably fewer globular inclusions

Fatty acid and simple lipid analysis

A partial total ion chromatogram of saponified extract from LM is given in Fig. 6. FAMEs are labelled numerically according to standard nomenclature *X*:*Y*n-*Z*, where *X* is the number of carbon atoms in the chain, *Y* is the number of double bonds present, and *Z* is the position of the first double bond from the methyl end to the carbonyl end. Other compounds of interest are assigned alphabetically. The occurrence and abundance of fatty acids are given in Table 1. Among the saturated straight chain fatty acids (SATFA), 16:0 was the most abundant, accounting for 65% and 53% of the total SAFTA in LM and NLM respectively. SATFA ranged from C_{12} to C_{24}, with an even-over-odd predominance. Monounsaturated fatty acids (MUFA) consisted of even-chain fatty acids ranging from C_{16} to C_{24}. Unsaturation on the ninth carbon from the methyl end dominated, and C_{18} MUFA were the major form (48% total MUFA), but there was also a relatively high abundance of 20:1n-9. MUFA were approximately twice as abundant in LM as in NLM. Polyunsaturated fatty acids (PUFA) were the second most abundant fatty acid class and primarily consisted of C_{20} PUFA (89% of the total PUFA), with 20:4n-6 accounting for 49% of the total PUFA in LM. There was a greater abundance of PUFA in LM than in NLM. Small amounts of methyl-branched (including iso- and anteiso-) fatty acids were identified. Thus, the major fatty acids in LM and NLM were 16:0 (30.7 and 23.9% respectively), 20:4n-6 (14.3 and 15.6%) and 20:5n-3 (6.9 and 8.6%) (Table 1).

A range of other lipids was also identified in maerl (Table 2). The diterpene alcohol phytol (3,7,11,15-tetramethyhexadec-2*E*-enol), which is the ester-linked side chain of chlorophyll *a*, and its degradation product phytanic acid (3,7,11,15-tetramethyhexadecanoic acid) were observed. The sterols cholesterol (3β-cholest-5-en-3-ol), β-sitosterol (Stigmast-5-en-3-ol) and stigmasterol (5,22-Stigmastadien-3β-ol) were identified as the major sterol components of maerl, with cholesterol accounting for 89% of the total sterols in LM. Steroid or sterol biosynthetic precursors and metabolites were also identified, namely squalene (2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene) and cholest-4-en-3-one. The monoacylglycerols monopalmitin (2,3-dihydroxypropyl hexadecanoate) and monostearin (2,3-dihydroxypropyl octadecanoate) were found in significant amounts in LM and NLM (monostearin could not be quantified due to coelution with disaccharides).

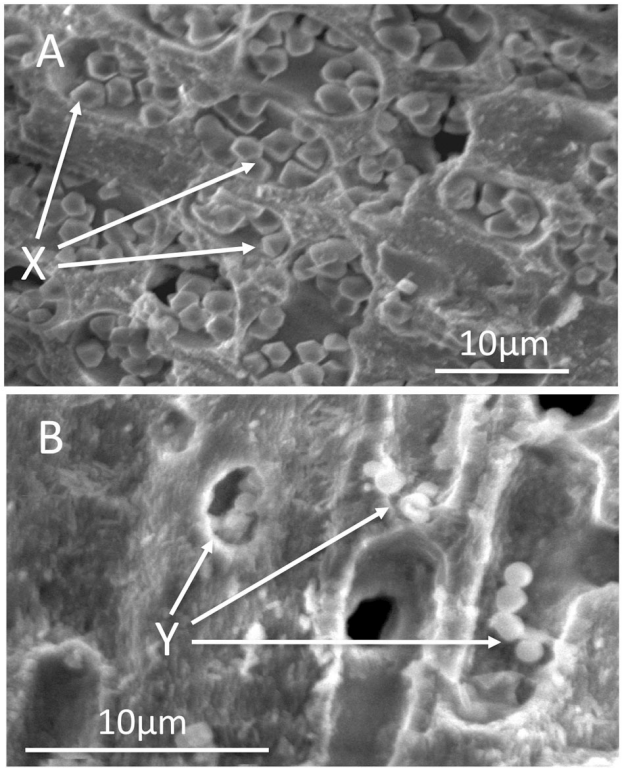

Fig. 5. SEM image of non-living maerl showing (A) globular inclusions with apparent angular morphology (X) and (B) 3.3 µm pore structures and abundant bacterial (≤1 µm) cocci- or streptococci-shaped structures (Y)

Low molecular weight carbohydrate analysis

In the present study, a preliminary analysis of the solvent extractable LMWC was carried out. The partial m/z 204 extracted ion chromatogram (EIC) silylated total extract from LM is given in Fig. 7, and the occurrence and abundance of LMWC are given in Table 2. Absolute identities of di- and trisaccharides could not be assigned due to high similarity in mass spectra and a lack of commercial standards. The most abundant compounds were a range of mono-, di- and trisaccharides, and compound occurrences were very similar for both LM and NLM. The α- and β-anomers of D-galactose were the major monosaccharides in both samples, with other monosaccharides, such as D-arabinose, xylulose, D-fructose and D-altrose, present in much lower abundance. A complex range of disaccharides was observed, with common disaccharides, such as sucrose and maltose, absent. A high abundance of a trisaccharide, tentatively assigned as galactotriose (Brokł et al. 2009), was also identified, in addition to a series of unresolved trisaccharides (m/z 204 base peaks and strong m/z 361 and m/z 217 peaks). Floridoside (2-O-glycerol-α-D-galactopyranoside) and isofloridoside (1-O-glycerol-α-D-galactopyranoside) were identified and found to be in high abundance in both LM and NLM (39.3 and 38.0 µg g^{-1} respectively) (Fig. 7, Table 2).

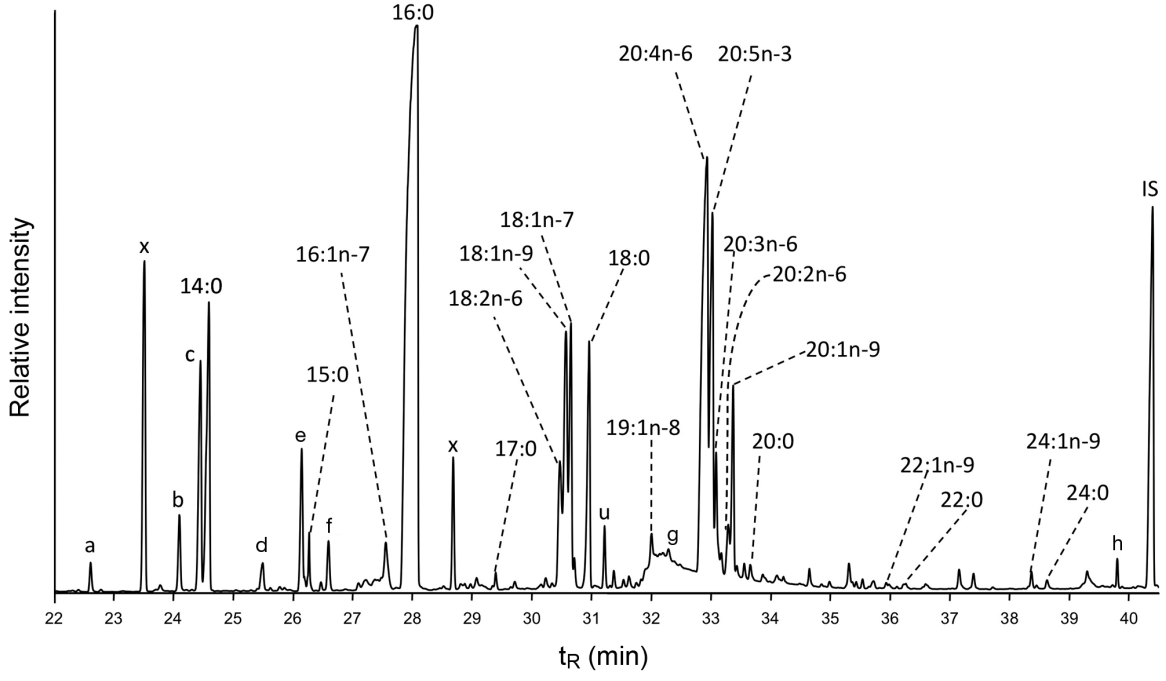

Fig. 6. Partial total ion chromatogram (TIC) of transesterified total extract from living maerl. Fatty acid methyl esters are numbered *X*:*Y*n-*Z*, where *X* is the number of carbon atoms in the chain, *Y* is the number of double bonds present, and *Z* is the position of the first double bond from the methyl end to the carbonyl end. a: tetradecanal, b: heptadecane, c; hexadecanal, d: octadecanal, e: 6, 10, 14-trimethyl-2-pentadecanone, f: phytol, g: squalene, IS: internal standard, u: unknown, x: contaminant

Table 1. Occurrence and concentration of fatty acids in living and non-living maerl. All fatty acids were identified as fatty acid methyl esters derivatives, and polar compounds were identified as trimethylsilyl-ether derivatives. MUFA double bond positions assigned by interpretation of DMDS adduct fragmentations (Nichols et al. 1986). SATFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, br-FA: branched fatty acids, DW: dry weight, nd: not detected, na: not applicable

	Compound		Molecular weight	Living maerl		Non-living maerl	
	Common name	Number	(g mol^{-1})	(µg g^{-1} DW)	% total fatty acid	(µg g^{-1} DW)	% total fatty acid
SATFA	Lauric	12:0	200	2.0	0.6	1.5	0.7
	Tridecanoic	13:0	214	1.5	0.5	1.1	0.5
	Myristic	14:0	228	20.1	6.0	11.8	5.5
	Pentadecanoic	15:0	242	7.8	2.3	4.6	2.2
	Palmitic	16:0	256	102.1	30.7	51.1	23.9
	Margaric	17:0	270	3.3	1.0	2.1	1.0
	Stearic	18:0	284	17.5	5.2	19.0	8.9
	Arachidic	20:0	312	5.3	1.6	4.1	1.9
	Heneicosanoic	21:0	326	1.3	0.4	1.3	0.6
	Behenic	22:0	340	1.9	0.6	1.8	0.8
	Tricosanoic	23:0	354	1.3	0.4	1.0	0.5
	Lignoceric	24:0	368	1.8	0.5	1.6	0.8
		Total		*165.9*		*101.0*	
MUFA	Palmitoleic	16:1n-7	254	8.3	2.5	4.1	1.9
	Vaccenic	18:1n-7	282	16.7	5.0	12.0	5.6
	Oleic	18:1n-9	282	14.2	4.3	6.8	3.2
	8-nonadecenoic	19:1n-8	296	6.8	2.0	nd	na
	Gondoic	20:1n-9	310	11.5	3.5	5.7	2.7
	Erucic	22:1n-9	338	1.3	0.4	1.8	0.8
	Nervonic	24:1n-9	366	2.6	0.8	1.4	0.7
		Total		*64.8*		*33.3*	
PUFA	Linoleic	18:2n-6	280	10.8	3.2	8.9	4.2
	Eicosadienoic	20:2n-6	308	6.2	1.9	2.7	1.3
	Dihomo-γ-linoleic	20:3n-6	306	8.6	2.6	6.7	3.1
	Arachidonic	20:4n-6	304	47.5	14.3	35.1	15.6
	Timnodonic (EPA)	20:5n-3	302	23.1	6.9	18.3	8.6
		Total		*96.2*		*71.7*	34.0
br-FA	Iso-pentadec.	i 15:0	228	1.7	0.5	1.2	0.6
	Anteiso-pentadec.	ai 15:0	228	1.5	0.5	1.1	0.5
	Branched-hexadec.	br 16:0	242	1.5	0.4	1.2	0.6
	Iso-heptadec.	i 17:0	256	2.4	0.7	1.2	0.6
	Anteiso-heptadec.	ai 17:0	256	2.3	0.7	nd	na
		Total		*9.4*		*4.7*	

DISCUSSION

Bulk physical and chemical composition

Aragonite and dolomite may not be present in significant amounts in *Lithothamnion corallioides* in Kingstown Bay (Fig. 2). While calcite is known to be the major mineral form for the Order Corallinales, minor components of aragonite and other minerals have been shown to be present under different microclimatic conditions (Bilan & Usov 2001, and references therein). Indeed, Blunden et al. (1977) reported that maerl is composed of ~80% calcite, 10 to 15% aragonite and <8% magnesium carbonate. Thus, the apparent lack of aragonite or high magnesium carbonate may be a characteristic of maerl at this site or may be related to variations in minor carbonate polymorph abundance with microclimatic conditions. Comparison of the LM and NLM X-ray diffractogram patterns indicates that little mineralogical alteration occurs in maerl upon death and early deposition. However, because ^{14}C-dating of the age differences between LM and NLM was not performed, and dating based on stratification was also not possible due to extensive tidal mixing of the maerl beds, no distinct conclusions can be drawn on the rate of mineralogical alteration at present. The IR analysis (Fig. 3) shows that overall there is a high degree of similarity in organic matter composition between LM and NLM, which indicates a minor contribution of organic matter compared to carbonate.

Table 2. Occurrence and concentration of non-fatty acid lipids and low molecular weight carbohydrates (LMWC) in living and non-living maerl. All LMWC identified as trimethylsilyl-ether derivatives. DW: dry weight, nq: not quantified, nd: not detected, na: not applicable

Compound	Molecular weight (g mol^{-1})	Concentration (µg g^{-1} DW) Living	Non-living
Lipids			
Tetradecanal	212	3.5	2.0
Hexadecanal	240	13.5	7.3
Octadecanal	268	9.2	5.5
Heptadecenal	252	4.7	1.7
Heptadecane	240	5.3	3.5
Glycerol	92	12.6	13.2
Phytol	296	6.1	13.7
Phytanic acid	312	7.1	2.1
Squalene	410	1.8	1.7
Monopalmitin	330	6.5	5.9
Monostearin	358	nq	nq
Cholesterol	386	22.9	19.2
β-sitosterol	414	2.9	1.3
Stigmasterol	412	1.0	1.0
Cholest-4-en-3-one	384	1.0	1.0
Monosaccharides			
α-Galactose	180	26.9	19.8
β-Galactose	180	15.5	12.8
α-arabinose	150	2.6	1.1
β-arabinose	150	2.1	1.5
D-Xylulose	150	2.1	0.4
α-Fructose	180	4.1	2.1
β-Fructose	180	4.6	2.2
L-Altrose	180	6.6	3.9
Total		*64.5*	*43.8*
Sugar alcohols			
Xylitol	152	2.3	nd
Arabitol	152	0.8	0.4
Ribitol	152	1.9	1.0
Mannitol	182	6.1	3.8
Galactitol	182	1.3	1.0
Total		*12.4*	*6.2*
Disaccharides	na	225.5	192.3
Trisaccharides	na	66.2	38.8
Floridoside	254	39.3	38.0
Isofloridoside	254	21.1	27.0

Globular inclusions

Globular inclusions (Fig. 4) were found in SEM analysis and have been reported previously in calcified algae, but their exact identity has not been established. They have been proposed to be chloroplasts or plastids (Borowitzka et al. 1974, Ghirardelli 2002), endophytic green algae (Alexandersson 1977), endophytic bacteria (Garbary & Veltkamp 1980) or starch grains (Wilks & Woelkerling 1994, Blunden et al. 1997). While these structures appear to be spheroid in shape and of similar size to bacteria (~3 to 4 µm in diameter), the observed structures possess an apparent angular, rather than spheroid, morphology (Fig. 5A, 'X') and are considerably larger and of different morphology than bacteria that were found in maerl samples (Fig. 5B, 'Y'). In addition, bacterial fatty acids biomarkers, such as iso- and anteiso-methyl-branched alkanoic acids (e.g. Zelles 1999), were not found in high abundance in maerl, and there was little variation between LM and NLM, suggesting that extensive colonisation by endophytic bacteria of NLM is not occurring. These results suggest that these globular inclusions are not bacteria.

Fatty acid and simple lipid composition

Assessing the fatty acid composition of algae is very useful in studies related to marine organic matter cycling, ecology, chemotaxonomy (e.g. Volkman 2006) and applied research (e.g. food, biomedical and biofuels research) (Zhukova & Aizdaicher 1995, Bigogno et al. 2002). In addition, the fatty acid composition of algae has been shown to be affected by changes in environmental conditions, such as temperature, light intensity or pH, and can be linked to growth rate (Cohen et al. 1988). The fatty acid content was similar for LM and NLM, but there was an overall decrease in abundance in NLM (Table 1). Overall, the fatty acid composition of maerl was found to be similar to that previously reported in many marine algae, where 16:0 and 20:5n-3 (Volkman et al. 1999) are the major fatty acids. However, it is noteworthy that 20:4n-6 is the major PUFA reported here, which may be a specific characteristic of *Lithothamnion corallioides* in Kingstown Bay. Fleurence et al. (1994) demonstrated that 20:4n-6 and 20:5n-3 were major fatty acids present in similar proportions in red macroalgae. Khotimchenko et al. (2002) showed that 20:5n-3 and 20:4n-6 were major fatty acids in all red algal species investigated, with proportions varying considerably but generally with 20:5n-3 being dominant. In contrast, the proportions of 20:4n-6 and 20:5n-3 were in similar in brown algae, and both fatty acids were present in low abundance in green algae. Graeve et al. (2002) investigated the fatty acid composition of marine arctic and antarctic macroalgae and showed that 16:0 and 20:5n-3 were the primary fatty acids and 20:4n-6 was of relatively minor abundance. However, the authors also noted that the species *Phycodrys rubens* and *Delesseria lancifolia* exhibited 20:4n-6 as a major

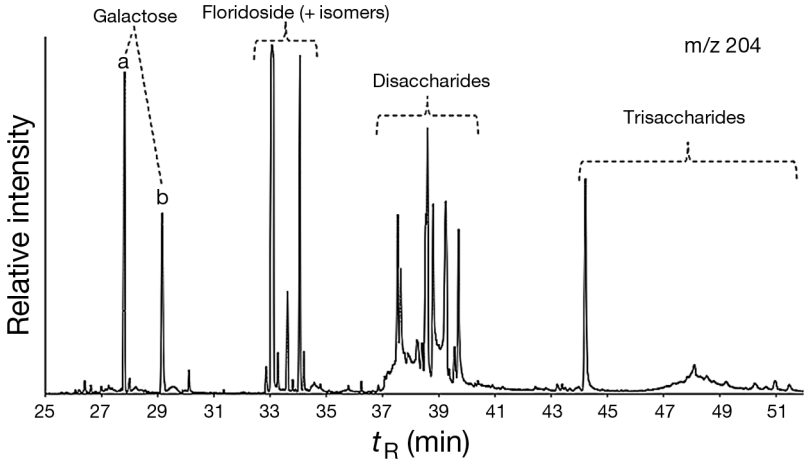

Fig. 7. Partial extracted ion chromatogram (m/z 204) of silylated extract from living maerl. t_R: retention time

fatty acid. It is of note that a contribution of fatty acids from colonised bacteria is possible, but because the occurrence of bacteria appears to be minor (Fig. 5), the contribution of bacterial fatty acids is likely also minor. The ratio of n-6/n-3 fatty acids is ~3 for maerl in the present study, and ratios <10 have been shown to be appropriate for dietary supplements to help prevent inflammatory, cardiovascular and nervous system disorders (Sánchez-Machado et al. 2004, van Ginneken et al. 2011). Thus, the proportions of PUFA found here are different than those of most investigated red algal species and may be species specific, and their high abundance and relative proportions indicate the potential of maerl as a dietary supplement. Long-chain saturated (C_{14}, C_{16} and C_{18}) and monounsaturated (C_{17}) aldehydes were found in relatively high abundance in both LM and NLM and are similar to those previously reported for red coralline algae (De Rosa et al. 1995) and various seaweeds (Kajiwara et al. 2007). The results presented here highlight distinct profiles, in particular PUFA, sterol and long-chain aldehyde abundance, which are apparently preserved upon death and thus may be of use from a chemotaxonomic perspective in ecological studies and organic matter cycling studies (e.g. Volkman 2006).

LMWC composition and occurrence of floridoside in maerl

Carbohydrates are common structural and storage compounds in terrestrial and marine organisms and are the primary form of photosynthetically assimilated carbon in the atmosphere (Cowie & Hedges 1984). They can be readily identified and quantified as trimethylsilyl ethers using GC-MS (Medeiros & Simoneit 2007, Füzfai et al. 2008). The α- and β-anomers of D-galactose were the major monosaccharides in LM and NLM, and complex range of disaccharides was observed, likely composed of galactose monomers (Table 2). Sulphated galactans, such as agars or carrageenans, are the most common polysaccharides extracted from red algae, are typically composed of repeating units of agarobiose or carrabiose disaccharides (Bilan & Usov 2001, Barsanti & Gualteri 2006) and are likely the dominant disaccharides found in maerl also.

Floridoside is commonly found in red algae and is thought to be the primary photosynthetic reserve product in most Rhodophyta (Impellizzeri et al. 1975, Raven et al. 1990, Karsten et al. 1993). Floridoside plays an important role in carbon storage, transport and assimilation and also in osmotic regulation (Weïwer et al. 2008). It is known to be a precursor in the synthesis of cell-wall polysaccharides in the red microalga *Porphyridium* sp. (Li et al. 2002). Variation in the abundance of floridoside and its isomers has been linked to diurnal and seasonal cycles (Karsten et al. 1993, Meng & Srivastava 1993). Interestingly, floridoside has recently been highlighted as a potentially important new anticomplementary agent for use in therapeutic complement depletion (Courtois et al. 2008), as a potentially health-promoting prebiotic foodstuff (Ishihara et al. 2010), as an antioxidant (Li et al. 2010) and in conjunction with isethionic acid as a novel bacterial quorum-sensing inhibitor (Liu et al. 2008). Thus, in light of the recent findings highlighting Ireland's significant maerl resource (De Grave & Whitaker 1999) and the apparent abundance of PUFA and floridoside, there is a clear potential for the commercial exploitation of maerl for biomedical, biotechnology and pharmaceutical industries. NLM has been shown to possess similar concentrations of floridoside (and PUFA) and may therefore be a more appropriate option for commercial exploitation.

Acknowledgements. We thank the Geological Survey of Ireland, the Irish Research Council for Science, Engineering & Technology (IRCSET), INFOMAR (Integrated Mapping for the Sustainable Development of Ireland's Marine Resources), the Irish Environmental Protection Agency, the Science Foundation of Ireland and QUESTOR for funding

this research. We also thank B. Twamley (Dublin City University) for SEM training and B. O' Connor (AQUAFACT Services) for benthic sampling and advice. We thank 3 anonymous reviewers for their helpful comments.

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Editorial responsibility: Christine Paetzold, Oldendorf/Luhe, Germany

Submitted: July 21, 2011; Accepted: March 16, 2012
Proofs received from author(s): May 17, 2012