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AN INVESTIGATION INTO ^{14}C OFFSETS IN MODERN MOLLUSK SHELL AND FLESH FROM IRISH COASTS SHOWS NO SIGNIFICANT DIFFERENCES IN AREAS OF CARBONATE GEOLOGY

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ABSTRACT. Our ability to reliably use radiocarbon (^{14}C) dates of mollusk shells to estimate calendar ages may depend on the feeding preference and habitat of a particular species and the geology of the region. Gastropods that feed by scraping are prone to incorporation of carbon from the substrate into their shells as evidenced by studies comparing the radiocarbon dates of shells and flesh from different species on different substrates (Dye 1994; Hogg et al. 1998). Limpet shells (*Patella sp*.) are commonly found in prehistoric midden deposits in the British Isles and elsewhere, however these shells have largely been avoided for radiocarbon dating in regions of limestone outcrops. Results from limpets (*Patella vulgata*) collected alive on limestone and volcanic substrates on the coasts of Ireland indicate that the shells were formed in equilibrium with the seawater, with no significant ^{14}C offsets. Limpets collected from the east coast of Northern Ireland have elevated ^{14}C due to the output of Sellafield nuclear fuel reprocessing plant. In all locations, the flesh was depleted in ^{14}C compared to the shells. The results will have an important consequence for radiocarbon dating of midden deposits as well as the bone of humans and animals who fed on the limpets.

KEYWORDS: limestone, limpet, mollusk, radiocarbon.

INTRODUCTION

Marine mollusk shells are commonly found in prehistoric middens across Ireland, having likely been an important dietary component of many prehistoric communities (Murray 2007). Limpets, periwinkles, and mussels all were exploited by prehistoric humans and their shells are widespread in archaeological sites across northern Europe (Gutiérrez-Zugasti et al. 2011). Shell middens have the potential to provide information on resource management and population movement, as well as past environments (Alvarez et al. 2011). Although mollusk shells provide carbon-rich material that can be measured for ^{14}C content, accurate chronological control of prehistoric events requires knowledge of any in-built age in the carbon source used for shell construction. Equally, knowledge of any in-built age in the flesh of the limpets will affect the dating of bones, where limpets have been a dietary component (Milner 2006; Meiklejohn and Woodman 2012).

For shell building, mollusks obtain carbon from two sources, dissolved inorganic carbon (DIC) in sea water, and organic carbon from within the marine food chain. The proportion of carbon derived from metabolic sources may depend upon a number of factors, including habitat and the way that individual mollusk species feed (Tanaka et al. 1986; Dettman et al. 1999). In some coastal areas, metabolic carbon originating from terrestrial plants and soils also could also be incorporated in mollusk shells. Feeding preference and habitat, on substrate containing old carbon such as limestone rock, can result in an apparently old age for both mollusk shell and flesh (Dye 1994). Subsequent research has avoided areas with limestone geology on the assumption that old carbon is incorporated into the samples. From the point of view of archaeological sites, humans or animals feeding on mollusks also can incorporate this carbon into bone collagen. In this way, determining the existence of such an age offset and its size is an important aspect of using ^{14}C for dating bones from coastal environments as well as shells.

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It is necessary to consider the biology of the mollusks to understand how geological carbon could potentially be taken up into the bodies and shells. *Patella vulgata*, also known as the common limpet, is a microphagous herbivore gastropod found in the intertidal zones of rocky shores in northwestern Europe. The powerful radula (teeth-like structure in mollusks) of this species of *P. vulgata* is capable of gouging deeply into the substrate to remove microalgae as well as other larger objects (Hawkins et al. 1989). The same mechanism, possibly combined with chemical dissolution using mucus, is used for creating the depression scar on which the *P. vulgata* makes its home (Lindberg and Dwyer 1983). *P. vulgata* usually feeds during daytime or nocturnal high waters and travels an average of 0.4 m from its home scar, returning before exposure occurs at low tide. Even if relocation is necessary, they do not travel more than 2 m to a new home (Hartnoll and Wright 1977). This would suggest that an individual is usually grazing on one type of substrate during its lifetime. In contrast, mussels (*Mylitus edulis*) attach to the substrate and siphon water to filter out plankton and microscopic organisms. Importantly for this study, Ascough et al. (2005) also have shown that the feeding and habitat behavior of *P. vulgata* and *M. edulis*, from a coastline with no geological carbon influence, did not have a significant influence on ^{14}C activity in their shells.

The coast of Ireland lies between the Atlantic Ocean to the west and the Irish Sea to the east. Outcrops of limestones are common and, in the north, are overlain by extensive basalts (GSNI 2018). Sellafield, on the Cumbrian coast in the northeast of the Irish Sea, is a nuclear reprocessing and decommissioning complex that processes all forms of radioactive waste. Normally we would expect the ^{14}C of the Irish Sea to be similar to that of the "global" ocean based on measurements from pre-nuclear testing shells (Harkness 1983; Blake 2005; Butler et al. 2009). However, radioactive waste including ^{14}C is discharged to the aquatic environment as low-level waste, primarily as DIC. This ^{14}C mixes with the existing ^{14}C in the seawater and is dispersed across the Irish Sea with the currents (Muir et al. 2016) and is incorporated into the shells of mollusks. The levels of concentration of discharged ^{14}C are monitored and published in an annual report as activity in Bequerels (Bq). In 2007, a total of 4.7 TBq of ^{14}C was discharged into the Irish Sea (Nuclear Decommissioning Authority 2007) and in 2017, a total of 3.6 TBq of ^{14}C was discharged (Nuclear Decommissioning Authority 2017).

In this paper we measure the ^{14}C of shells and flesh of intertidal limpets (*P. vulgata*) from both volcanic and limestone rocks at six sites from around the Irish coast to determine variation in mollusk ^{14}C content. We are particularly interested in documenting any species-specific, shell-flesh, and granite/basalt-limestone ^{14}C offsets. It was expected that the discharge of ^{14}C into the Irish Sea from the Sellafield nuclear reprocessing site located on the opposite shore would accentuate the difference between ingested limestone and dissolved inorganic carbon of the seawater in specimens from the west coast of Northern Ireland and help identify the need for reservoir corrections.

METHODS

Sample Collection

Five individual limpets (*Patella vulgata*) were collected living on intertidal limestone and basalt rocks sites on the east coast of Northern Ireland (EC) at Portmuck and The Gobbins, Islandmagee, in October 2007. Five limpets were also collected living on limestone and granite rocks on the west coast of Ireland (WC) at Black Head and near Loughaunbeg, Galway Bay. Analyses were completed but the results were not published at that time. Further samples were collected in July and September 2018 from the north coast (NC) at

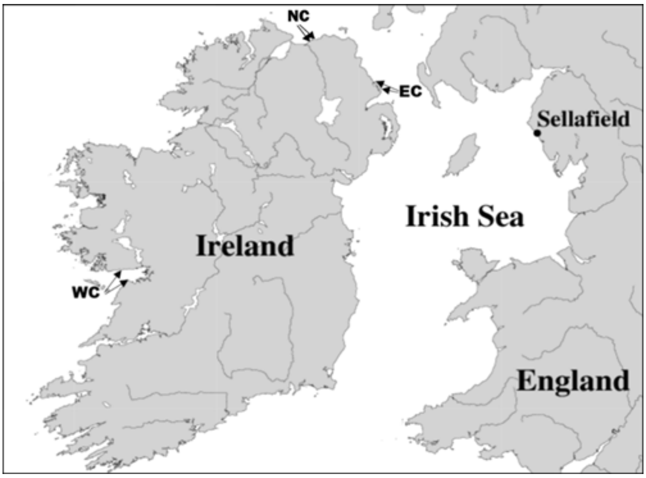

Figure 1 Location map of sample collection sites.

Whiterocks, Portrush, and Rinagree Point, Portstewart. The locations of the sample sites are shown in Figure 1 and geological substrate is included in the results tables. A full list of samples collected can be found in Tables 3 and 4 in the appendix. For comparison, samples of mussels (*M. edulis*) were also collected from two of the sites, as *M. edulis* is a filter-feeding mollusk and should not be affected by substrate type.

Radiocarbon Analyses

Mollusk individuals were heated in Milli-Q® water until the flesh easily separated from the shell. The digestive organs were identified under a low-powered binocular microscope, then cut away from the remaining flesh and discarded, ensuring that the entire digestive tract was removed. The flesh was treated with 4% HCl for 1 hr at 80ºC, rinsed five times with Milli-Q® water, dried overnight at 70ºC, and ground with mortar and pestle until all material passed through a 250-μm sieve to ensure homogeneity. A subsample (~100 mg) of each shell was etched in 1% HCl (2 mL per 100 mg) overnight to remove surface carbonates, rinsed five times with Milli-Q® water, dried overnight at 70ºC, and coarsely ground with a mortar and pestle.

Subsamples of mollusk flesh for ^{14}C analysis were individually loaded into quartz sample tubes with 30 mg of pre-combusted CuO and a strip of Ag foil then sealed under vacuum. Sealed tubes were combusted to CO_2 at 900°C for at least 4 hr. Approximately 12 mg of each pretreated shell was weighed into 4 mL Exetainer® vials (LabCo, Inc.), evacuated, injected with 1.5 mL of concentrated (85%) phosphoric acid to hydrolyze the shell to CO_2, and heated at 80ºC for 20 min or until all bubbling ceased.

Samples were converted to graphite on an iron catalyst using the hydrogen reduction method (Vogel et al. 1987) and the $^{14}C/^{12}C$ ratio and $^{13}C/^{12}C$ were measured by accelerator mass spectrometry at the ^{14}CHRONO Centre, Queen's University Belfast. The radiocarbon age and one standard deviation were calculated using the Libby half-life of 5568 years following the conventions of Stuiver and Polach (1977). The ages were corrected for

Table 1 ^{14}C-AMS measurements of *P. vulgata* from the coast of Ireland presenting the weighted means and standard deviations of the groups of measurements. The P values represent the analysis of differences between *P. vulgata* on limestone and basalt/granite substrates.

Limpet samples	F^{14}C shell	Std dev	*P* value	F^{14}C flesh	Std dev	*P* value
East coast basalt	1.575	0.0390	0.524	1.446	0.0416	0.008
East coast limestone	1.525	0.0924		1.368	0.0198	
West coast granite	1.063	0.0039	0.999	1.070	0.0066	0.514
West coast limestone	1.063	0.0062		1.067	0.0046	
North coast basalt	1.121	0.0219	0.412	1.146	0.0125	0.001
North coast limestone	1.113	0.0168		1.052	0.0231	

Table 2 ^{14}C-AMS measurements of *M. edulis* from the coast of Ireland presenting the weighted means and standard deviations of the groups of measurements. The P values represent the analysis of differences between *P. vulgata* and *M. edulis* on limestone and basalt/granite substrates.

Mussel samples	F^{14}C shell	Std dev	*P* value	F^{14}C flesh	Std dev	*P* value
East coast limestone	1.534	0.0438	0.708	1.391	0.1187	0.259
West coast limestone	1.066	0.0042	0.441	1.068	0.0035	0.762

isotope fractionation using the AMS measured δ^{13}C (not given) which accounts for both natural and machine fractionation. ^{14}C data are reported as F^{14}C (Reimer et al. 2004).

A statistical analysis of the groups of measurements, using one-way analysis of variance (ANOVA), was carried out between the sets of limpets at each site as well as testing the *P. vulgata* and *M. edulis* samples as three groups at the east and west coast sites. ANOVA is a test of differences with the null hypothesis being that two sets of data are the same and can be used to test more than two groups of samples together. In this study we are aiming to accept the null hypotheses, so a *P* value of above 0.05 is needed to be confident that there are no statistical differences between data sets.

RESULTS

A summary of the F^{14}C results are presented in Tables 1 and 2 and Figure 2. A full list of the results is presented in Tables 3 and 4 in the appendix. A significant difference between east coast compared to north and west coast results is apparent, with the east coast results showing an enrichment of ^{14}C. A smaller enrichment of ^{14}C is also apparent in the north coast results.

The results from the *P. vulgata* shell samples indicate that there is no statistical difference between the volcanic and limestone locations. The results from the west coast shell samples indicate no statistical difference (ANOVA $F_{1,8} = 0.1.69\text{E-}06$, $P = 0.999$). The results from the east and north coast shell samples also indicate no statistical difference between, but with lower confidence (ANOVA $F_{1,8} = 0.444$, $P = 0.52$ and ANOVA $F_{1,8} = 0.748$, $P = 0.412$). The results from the flesh samples indicate no statistical difference between volcanic and limestone locations on the west coast (ANOVA $F_{1,8} = 0.466$, $P = 0.514$) but a significant difference on both east and north coasts (ANOVA $F_{1,8} = 15.40$, $P = 0.004$ and ANOVA $F_{1,8} = 49.09$, $P = 0.0001$). *P* values and standard deviations are included in Table 1. All the

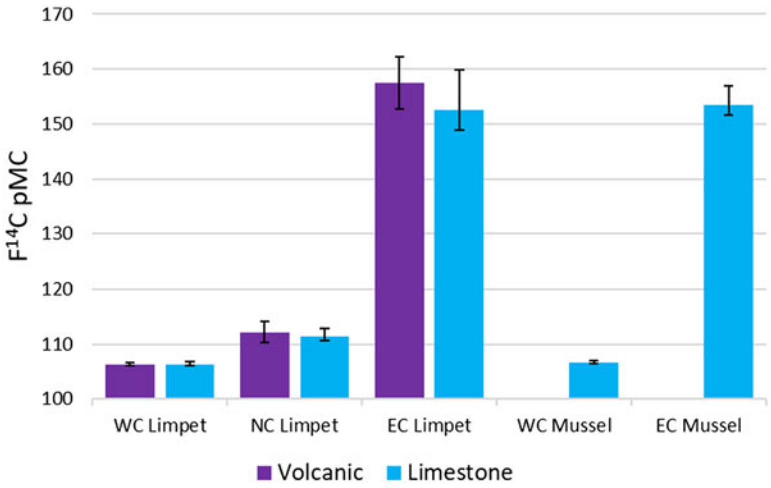

Figure 2 ^{14}C activity of shell and algae samples as per cent modern carbon (pMC) with an associated error derived from the standard error of each group to express the natural variability in the shells.

flesh samples are depleted in ^{14}C compared to the shell samples, although the shell and flesh from the west coast are not statistically different (ANOVA $F_{1,8} = 3.88$, $P = 0.084$).

Adding the results for the *M. edulis* samples also indicates that there is no statistical difference between the two species. Statistical analysis of the three groups of west coast limestone shell samples (ANOVA $F_{2,12} = 0.876$, $P = 0.441$) and three groups of east coast limestone shell samples (ANOVA $F_{2,12} = 0.355$, $P = 0.708$) indicates no statistical difference between species. The three groups of flesh samples also indicate no statistical difference on the west coast (ANOVA $\mathrm{F}_{2,11} = 0.278$, $\mathrm{P} = 0.762$) or on the east coast (ANOVA $F_{2,12} = 1.51$, $P = 0.259$). *P* values and standard deviations are included in Table 2.

As a comparison to data collected from Irish Sea shells by Muir et al. (2016), the $F^{14}C$ results were converted to ^{14}C activity in Bq kg^{-1} (Mook and van der Plicht 1999) and are presented in Figure 3. An existing "background" value of 249±1 Bq kg^{-1} C has been defined using shells collected on the west coast of Ireland (Muir et al. 2016), representing natural production of DIC and atmospheric fallout. The west coast *P. vulgata* samples match this background value well. The east coast samples are also consistent with the enrichment of marine biota (flesh) samples due to Sellafield shown in the Muir et al. study, where values ranged between 275±1 Bq kg^{-1} C to 287±1 Bq kg^{-1} C. Actual levels of ^{14}C enrichment vary with location and the level of discharge from Sellafield.

DISCUSSION

It is evident that the west coast samples are the only ones that do not show ^{14}C enrichment and provide the most stable results. The east coast locations are in the northern half of the Irish Sea, where ^{14}C discharges from Sellafield have a large effect. The north coast locations were assumed to be out of range of the Sellafield discharge, but the slight enrichment indicates that there is some influence, possibly from currents being funnelled between Rathlin Island and the north coast (Pingree and Griffiths 1980; Hill et al. 1997). The high standard

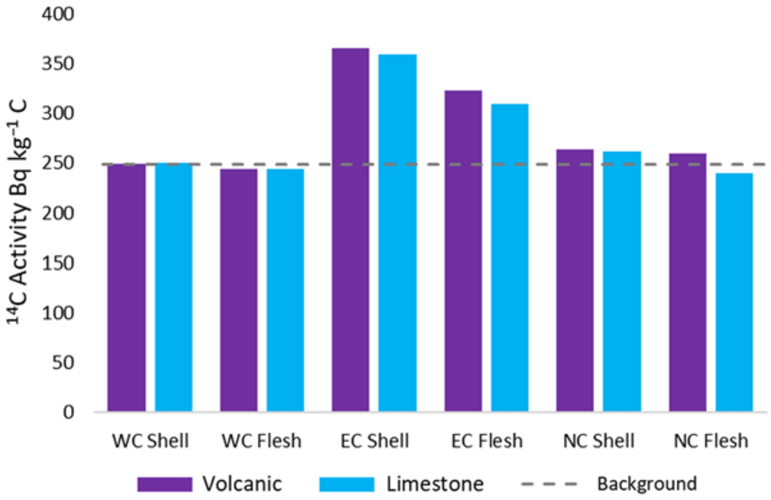

Figure 3 ^{14}C activity in *P. vulgata* as Becquerel per kilogram (Bq kg^{-1} C).

deviations for the east and north coast samples could be explained by the varied discharge of ^{14}C from Sellafield and, therefore, varied concentrations of ^{14}C in the seawater DIC. The live samples that were collected were not aged, as the growth rings were not easily distinguishable, so uptake of Sellafield derived ^{14}C may not be uniform across all the samples.

Statistically, the results indicate that there is no difference in the ^{14}C activity of the shells of *Patella vulgata* living on limestone and those living on other non-carbonate substrates. This, along with the comparison to the filter feeder results from the *Mytilus edulis*, suggests that this species does not metabolise carbon from the limestone at these locations. The uptake of Sellafield ^{14}C in the east and north coast samples highlights the influence of seawater DIC in both shell and flesh. Although Tanaka et al. (1986) and Dettman et al. (1999) concluded that metabolic carbon contributed up to 40% of the carbon in shell precipitation, Gillikin et al. (2006) revised this figure to approximately 10% depending on the species. The results of this study suggest that, for *P. vulgata* on the Irish coastline, this figure could be lower. The depletion in ^{14}C activity of the limpet flesh is unexplained but may be due to input of older terrestrial organic carbon, possibly from fossil fuel-based compounds especially at the east coast site. On the west coast, the flesh is not significantly depleted compared to the shell. Further study of mollusks from this coast, in areas where there is no anthropogenic input, would provide more data to investigate any differences between shell and flesh.

The biology and anatomy of the digestive system of *P. vulgata* may be key to understanding the results of this study. Inorganic and organic particles are sorted in the limpet digestive system. Inorganic particles are removed by ciliary action and only organic particles are directed to the digestive gland where most of the digestion occurs (Figure 4a). Absorption only occurs in the digestive gland and not in the rest of the digestive system as it would in some other mollusk species. The lowest pH value for the limpet digestive system is 5.4 (Yonge 1925). The solubility of limestone is very low at this pH value, but some dissolution may be possible. Carbonic anhydrase, an enzyme that catalyses the reversible CO_3 to CO_2 reaction, is present in limpet tissues but is not known to be secreted to the stomach (Rao 1975; Lindberg and

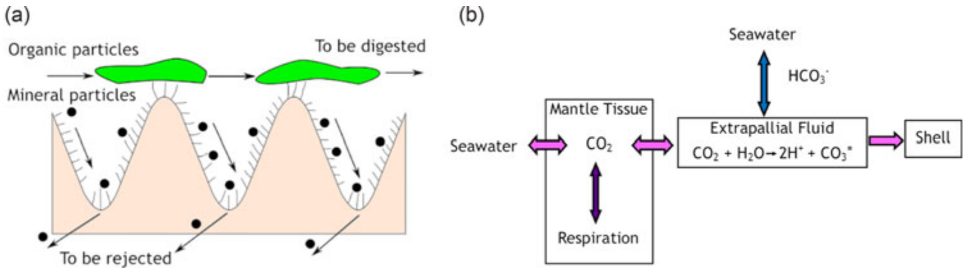

Figure 4 (a) sorting area in the *P. vulgata* digestive system (b) model of likely transport routes for inorganic carbon, based on figure from McConnaughey and Gillikin (2008).

Dwyer 1983). The uptake of CO_2 for shell formation is mainly by diffusion or fluid exchange between seawater and the extrapallial fluid (the fluid in the space between the body and shell where mineral precipitation takes place). CO_2 uptake for tissue formation is mainly from metabolic respiration but also by diffusion from seawater (McConnaughey and Gillikin 2008). A model for the uptake of CO_2 is presented in Figure 4b. This model explains how the seawater DIC has the greatest influence on mollusk shell as well as a strong influence on the flesh and this is supported by the results of this study. The east coast results, reflecting the Sellafield enrichment, show that the CO_2 in the mantle tissue is not only derived from respiration, but also from diffusion from seawater. The greatest influence of Sellafield ^{14}C is in the shell, as the carbon used for the shell building is mainly derived from seawater.

CONCLUSION

This study aimed to determine if off-sets from seawater ^{14}C were required when dating the shells of *Patella vulgata* that have fed on carbonate limestone substrates. Previous studies have suggested that foraging gastropods would metabolise old carbon, which would then be incorporated into their shells. The ^{14}C of shells and flesh from P. vulgata, which fed on either substrate, were measured. The results indicate that the majority of shell carbon comes from ambient seawater DIC. Any effects on the ^{14}C of *P. vulgata* from foraging on limestone are insignificant with respect to isotopic analysis. Shells from the east coast of Ireland are enriched by Sellafield derived carbon, causing higher variances within sample groups. However, these samples also indicate that there is no statistical difference between *P. vulgata* that fed on carbonate or volcanic substrates. The reason for this may be because of the primitive digestive system of *P. vulgata*. This study is relatively small, in respect to sample size, and a larger study would be needed to confirm these results. The study also indicated that the flesh is depleted in ^{14}C compared to the shell. A larger study would provide more data so that this finding could be investigated further.

The shells of this species that are often found in archaeological sites should be suitable for radiocarbon dating, even if they are thought to be from carbonate geology, although further analysis of archaeological samples would be required to confirm these findings.

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APPENDIX

Full Results of AMS Measurements

Table 3 ^{14}C-AMS measurements of *P. vulgata* from the coast of Ireland.

	Shell			Flesh		
Sample ID	UB no.	$F^{14}C$	$F^{14}C\ \sigma$	UB no.	$F^{14}C$	$F^{14}C\ \sigma$
East coast basalt (The Gobbins)						
GO-L1	8984	1.6081	0.0031	8889	1.4397	0.0058
GO-L2	8985	1.5764	0.0028	8890	1.4820	0.0068
GO-L3	8986	1.6089	0.0048	8891	1.4528	0.0069
GO-L4	8987	1.4826	0.0082	8892	1.4786	0.0059
GO-L5	8988	1.5393	0.0032	8893	1.3787	0.0063
East coast limestone (Portmuck)						
PM-L1	8979	1.3906	0.0028	8884	1.3576	0.0056
PM-L2	8980	1.5940	0.0028	8989	1.3629	0.0042
PM-L3	8981	1.5530	0.0036	8886	1.3820	0.0069
PM-L4	8982	1.5850	0.0034	8887	1.3849	0.0039
PM-L5	8983	1.5463	0.0037	8888	1.3305	0.0067
West coast granite (Loughaunbeg)						
LB-L1	9665	1.0641	0.0038	9505	1.0600	0.0064
LB-L2	9666	1.0666	0.0024	9506	1.0772	0.0059
LB-L3	9667	1.0579	0.0026	9507	1.0676	0.0065
LB-L4	9668	1.0641	0.0037	9508	1.0687	0.0044
LB-L5	9669	1.0628	0.0037	9509	1.0769	0.0067
West coast limestone (Black Head)						
BH-L1	9670	1.0555	0.0026	9510	1.0746	0.0063
BH-L2	9671	1.0664	0.0023	9511	1.0662	0.0050
BH-L3	9672	1.0647	0.0034	9512	1.0663	0.0048
BH-L4	9673	1.0584	0.0022	9514	1.0602	0.0060
BH-L5	9674	1.0705	0.0023	9515	1.0695	0.0046
North coast basalt (Rinagree Point)						
RP-L1	38850	1.1101	0.0037	38855	1.1429	0.0036
RP-L2	38851	1.0877	0.0041	38856	1.1549	0.0034
RP-L3	38852	1.1321	0.0045	38857	1.1540	0.0042
RP-L4	38853	1.1399	0.0033	38858	1.1541	0.0040
RP-L5	38854	1.1358	0.0045	38859	1.1257	0.0038
North coast limestone (Whiterocks)						
WR-L1	38474	1.1113	0.0044	38484	1.0281	0.0024
WR-L2	38475	1.1234	0.0034	38485	1.0735	0.0023
WR-L3	38476	1.1102	0.0048	38486	1.0487	0.0026
WR-L4	38477	1.0836	0.0040	38487	1.0417	0.0025
WR-L5	38478	1.1241	0.0029	38488	1.0920	0.0043

Table 4 ^{14}C-AMS measurements of *M. edulis* from the coast of Ireland. * Insufficient sample recovered following pretreatment.

	Shell			Flesh		
Sample ID	UB no.	F^{14}C	F^{14}C σ	UB no.	F^{14}C	F^{14}C σ
East coast limestone (Portmuck)						
PM-M1	8974	1.4732	0.0035	8931	1.3076	0.0037
PM-M2	8975	1.5451	0.0040	8932	1.2541	0.0052
PM-M3	8976	1.5569	0.0043	8933	1.4920	0.0055
PM-M4	8977	1.5780	0.0036	8934	1.5218	0.0072
PM-M5	8978	1.5265	0.0047	8935	1.4854	0.0042
West coast limestone (Black Head)						
BH-M1	9675	1.0700	0.0023	9516	1.0658	0.0037
BH-M2	9676	1.0608	0.0021	9518	1.0642	0.0065
BH-M3	9677	1.0690	0.0021	9519	1.0731	0.0066
BH-M4	9678	1.0634	0.0021	9520	1.0707	0.0035
BH-M5	9679	1.0691	0.0025	*	*	*