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Ionizing radiation-induced DNA damage response identified in marine mussels, *Mytilus* sp.

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ABSTRACT

There is growing concern over the potential detrimental impact of ionizing radiation on natural biota. The mechanistic cause-and-effect impact of ionizing radiation has yet to be characterized in any aquatic species. Adopting an integrated approach, including radiochemical analysis of environmental samples, we evaluate molecular responses to ionizing radiation in the marine mussel, *Mytilus edulis*. These responses included analyses of *RAD51* mRNA expression, a gene involved in the repair of DNA double strand breaks, and induction of DNA strand breaks using the comet assay, in samples collected from a site impacted by low level ionizing radiation discharges. Based on activities of the radionuclides measured in sediment and mussel tissue at the discharge site, external and internal dose rates were low, at *ca.* 0.61 μGyh^{-1} and significantly lower than the generic (all species) "no effect" dose rate of 10 uGyh^{-1}, yet DNA strand breakage and *RAD51* mRNA expression were both altered.

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1. Introduction

Contaminants in the environment can detrimentally affect aquatic organisms. While we have some mechanistic knowledge regarding the sub-lethal, molecular level biological effects of certain classes of chemical contaminants, for instance metals and organic compounds (e.g. polycyclic aromatic hydrocarbons) we currently know relatively little about such effects of physical contaminants (e.g. ionising radiations). Evidence suggests that marine organisms are also susceptible to damage from physical contaminant sources including radiation, yet the corresponding knowledge for aquatic organisms about sub-lethal, molecular level biological effects in such cases is lacking.

Elevated activities, and biological effects, of ionizing radiation have been investigated in the marine environment and marine species respectively, using both environmental and experimental exposure regimes (summarised in Table 1). Current radioprotection studies of biota target evaluation of measures such as reproductive success and cytogenetic effects since these are considered to have the highest impact at population and ecosystem levels (Brechignac, 2003). Given that DNA is the most important target for the action of ionizing radiations, it is apparent that the elevated activities of alpha, beta and gamma-emitting radionuclides present in marine environments, particularly in contaminated sediments, could be associated with genetic damage with potential detrimental impacts on ecosystem quality (Jha, 2004, 2008; Jha et al., 2005; Hagger et al., 2005). Due to technical limitations (Dixon et al., 2002), much of the work to date that examines radiation damage in aquatic organisms has utilised the comet and micronucleus assays (Jha et al., 2005; Hagger et al., 2005; Jaeschke et al., 2011). These techniques are limited, however, as they only examine gross DNA damage, are not specific to ionizing radiation stress alone, and are subject to natural seasonal, sex, and age variation.

Using available information from the human health arena concerning the specific mechanisms of action of radiation-induced damage, it is possible to focus on DNA damage response pathways that are activated upon exposure to ionizing radiation. Subsequently it becomes possible to monitor the activities of key proteins involved in that response to assess the biological impact, which is the focus of current research in environmental activity (Pentreath, 2009). In this context, *RAD51* is one such protein that assists in the repair of DNA double strand breaks (DSBs) which are induced by ionizing radiation and is also highly conserved from yeast to humans (Thacker, 2005). Analysis of *RAD51* therefore allows an analysis of biological response following exposure to a source of physical stress, which induces DSBs, and, is also discrete

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Table 1
Summary of ionizing radiation levels and biological effects currently reported in the literature in marine organisms.

Ionizing radiation source	Organism	Exposure regime	Biological effects	Reference
Po-210 Cabo Frio Is., Brazil	*Perna perna*	0.155 Bqg^{-1} wet weight, 0.02 $mGyd^{-1}$	No increase in micronuclei frequency nor DNA strand breakage	Cheung et al., 2006
Ra-226	*Hediste diversicolor*	0.03–6.6 Bqg^{-1}	Uptake confirmed, no effect on oxygen radical scavenging parameters	Grung et al., 2009
Cs-137& tritiated water	*Ophryotrocha diadema*	7.3 Gyh^{-1}	Decrease in number of larvae and eggs produced	Knowles and Greenwood, 1997
Cs-137	*Neanthes arenaceodentata*	2 Gy dose 4 Gy dose	Increase in chromosomal aberrations Decrease in broodsize	Anderson et al., 1990
Cs-137	*N. arenaceodentata*	5–10 Gy dose 0.5 Gy dose	Decrease in broodsize Increase in embryo mortality	Harrison and Anderson, 1994a
Co-60	*N. arenaceodentata*	Chronic dose: 0.19–17 $mGyh^{-1}$; total dose 0.5–5 Gy	Increase in embryo mortality at highest dose. Increased number and % of abnormal embryos	Harrison and Anderson, 1994b
Tritiated water	*M. edulis* embryos	0.02–21.41 mGy	Dose dependent increase in sister chromatid exchange between 3.7 and 370 kBq/ml Increase in chromosomal aberrations at 3.7 kBq/ml	Jha et al., 2006
Tritiated water	*M. edulis*	12–485 μGyh^{-1} for 96 h	Increase in micronuclei frequency, DNA strand breakage	Jha et al., 2005
Tritiated water and glycine	*M. edulis*	79–122 μGyh^{-1} (HTO) 4.9 μGyh^{-1} (T-Gly)	Increase in micronuclei frequency	Jaeschke et al., 2011
Am-241, Cm-244, Pu-238, Pu-239, Po-240, Cs-137, K-40, French Coast	*Crassostrea gigas*	Field samples Highest values: 0.5 $Bqkg^{-1}$ dry weight Cs-137	No significant difference in mRNA expression of selected stress response genes (heat shock proteins, metallothionein, superoxide dismutase)	Farcy et al., 2007
I-131, Cs-134, Cs-137 Fukushima accident, 30 d after event	Forest and marine biota	210-4600 $mGyd^{-1}$; 2600 $mGyd^{-1}$ for benthic fish, molluscs, crustaceans	None measured	Garnier-Leplace et al., 2011

from single strand breaks (SSBs) caused by many classes of chemical contaminants that may also be present in the same environment. Oxidative stress and free radical production is also a single strand DNA damaging pathway. If two such single strand damage events happen in close proximity then these may, in theory [as stated in Jackson and Bartek (2009)], lead to double strand damage, but there is no literature to quantify or validate this theory. Induction of DSBs is biologically important since they give rise to chromosomal aberrations which are involved in the initiation and promotion of malignancy and could impair the reproductive success of the organisms, one of the important ecotoxicological parameters (Natarajan et al., 1993; Jha, 2008).

Herein, we describe isolation of the *Mytilus edulis* homolog of the DNA damage response *RAD51* mRNA. Using this sequence we develop a quantitative assay of *RAD51* mRNA expression in this sentinel species (i.e. the marine blue mussel) to investigate subcellular (DNA level) specific impact in environmental samples collected from site exposed to low levels of anthropogenic radionuclide input and a reference site. Data are compared and complemented with established generic DNA damage assessment approaches (i.e. comet assay) and with environmental activities of key anthropogenic and naturally-occurring radionuclides. The study therefore aimed to obtain a holistic picture of the biological responses in an ecologically relevant marine organism following exposure to ionizing radiation under field conditions.

2. Materials and methods

2.1. Sample collection

Reference mussels were collected at low tide near Brighton Marina (BM), U.K. (50°48′ N latitude, 00°05′ E longitude) in July 2010, kept in seawater and immediately brought to the laboratory. *M. edulis* samples were also collected from the Ravenglass Estuary (RE), U.K. (54°21′ N latitude, 03°25′ W longitude) in July 2010, a site known to receive historical and current inputs of a range of anthropogenic radionuclides discharged (under license) from the Sellafield nuclear facility, Cumbria, UK. Local gamma doses (at 1 cm distance from the sediment substrate) at the sample collection sites were determined using a Mini Instruments Environmental Meter type 6-80, and underlying sediments retrieved for subsequent radionuclide analysis.

2.2. Isolation of RAD51 mRNA sequence from M. edulis

Total RNA was extracted from approximately 30 mg of reference *M. edulis* gonadal tissue, using RNA isolation® (Roche Applied Science, U.K.) reagents following the manufacturer's protocol. cDNA was synthesized from 1 μg of total RNA using a Transcriptor First-Strand cDNA Synthesis System from Roche (Roche).

Based on a sequence of *RAD51* from fresh water mussels, *Dreissena polymorpha* (GenBank Accession No. AF508221), the following oligonucleotides were designed: upstream primer 5′ TGTCACACTCTGGCAGTCACCTG-3′ and downstream primer 5′ GGGGTCTGCAGAAAACATGGC-3′. For RT-PCR, 3 μl of the synthesized cDNA in 16.5 μl of water was mixed with 0.5 μl of 50 nM of each primer, 0.5 μl of bovine serum albumin, 0.5 μl of dimethyl sulfoxide, 0.5 μl of dNTP, 2.5 μl of 10×PCR buffer and 0.5 μl of 50×Advantage 2 Polymerase Mix (Clontech Ltd., U.K.). The PCR conditions consisted of an initial denaturation at 95 °C for 1 min, followed by 35 cycles of denaturation at 95 °C for 15 s, annealing at 48 °C for 15 s and extension at 68 °C for 1 min. An expected molecular size (441 bp) product was visualized by electrophoresis on 0.8% agarose gel and DNA purified using QIAquick Gel Extraction Kit (Qiagen Ltd., U.K.).

The purified PCR products were inserted into pCR®2.1 vector, cloned using *Escherichia coli* competent cells (Promega, U.S.A.), and three clones randomly selected and sequenced with reversed primer M13 (-29) (Eurofins Operon MWG, Germany). The consensus DNA sequence was analysed using BLAST and a sequence alignment obtained using ClustalW2.

2.3. Determination of radionuclide activities in mussel tissue and sediment samples

Mussel tissue from samples retrieved from BM and RE, and local sediment samples, were pooled and analysed for ^{90}Sr, Pu isotopes, ^{210}Po and a range of gamma-emitting radionuclides (including ^{137}Cs, ^{241}Am, ^{40}K, ^{238}U and daughter products), which are known to be the major anthropogenic and naturally-occurring radionuclides contributing to dose in the RE area (RIFE 14, 2009). Samples for gamma spectrometric analysis were counted on a well-type HPGe detector previously calibrated with a mixed nuclide standard of identical geometry. Strontium-90 activity was determined by measuring the in-growth of the ^{90}Y daughter using Cerenkov counting, following spiking with ^{85}Sr and pre-concentration by precipitation as an oxalate and purification using extraction chromatography. For Pu isotope analysis, an aliquot of the ignited sample was spiked with ^{242}Pu as a chemical yield monitor and fused at 1000 °C with lithium metaborate flux. The resultant glass was dissolved and the solution filtered. The Am and Pu were isolated from the solution and one another by anion exchange chromatography. $^{238,239,240}Pu$ activities were determined by alpha spectrometry. ^{210}Polonium activities were determined by alpha spectrometry following spiking with ^{209}Po, *aqua regia* extraction and autodeposition onto Ag discs.

2.4. Determination of DNA strand breaks using comet assay in mussels

The single cell gel electrophoresis (SCGE) or the comet assay was applied for the analysis of DNA damage and followed the method described elsewhere in detail (Jha et al., 2005; Cheung et al., 2006; Canty et al., 2009) with minor modification. Haemolymph samples were obtained from mussels collected from a reference site in Cornwall (Di et al., 2011) and exposed to a range of concentrations of hydrogen peroxide (0–500 μm) following which the cells were processed for the comet assay (Cheung et al., 2006; Canty et al., 2009; Di et al., 2011; Millward et al., 2012). Briefly, super-frost slides were coated with 1.5% normal melting agarose (NMPA, w/v) and left to air dry at least 24 h prior to the comet assay. Haemocyte (200 μl) which isolated from posterior adductor muscle was suspended in same volume of chilled PBS. The cell suspension was then centrifuged at 9600 × *g* for 2 min. The supernatant was removed and replaced with 200 μl 0.75% low melting point agarose (LMPA, w/v). The mixture was immediately pipetted as two drops of 100 μl microgel to precoated super-frost slides. Coverslips were placed over each drop and gels were allowed to set at 4 °C for 1 h. When the gels had solidified to form duplicated microgels, coverslips were gently removed and the slides were immersed for 1 h in cold (4 °C) lysis solution [2.5 M NaCl, 100 mM EDTA, 10 mM Tris base, 1% N-Lauroyl-sarcosine, 1% Triton X-100, 10% DMSO, pH = 10]. After the lysis period, slides were placed in a horizontal electrophoresis unit containing freshly prepared electrophoresis buffer [0.3 M NaOH, 1 mM EDTA, pH >13]. The DNA was allowed to unwind for 30 min before electrophoresis at 25 V, 260 mA for 30 min. The slides were removed from the electrophoresis tank and gently immersed in neutralization buffer [0.4 M Tris base, pH = 7.5] for 5 min and this step was repeated three times followed by rinsing with distilled water for 10 min and drying overnight. Finally, to visualize comets, 40 μl of ethidium bromide (20 μg ml^{-1}) stain was applied to each gel. Cells were randomly selected and measured by video capture and image analysis using Komet 5.0 software (Kinetic Imaging, Liverpool, U.K.) with 50 cells scored per microgel and % DNA in tail determined (Kumaravel and Jha, 2006).

2.5. Quantitative real-time PCR analysis of RAD51 mRNA expression in gonadal tissues of mussels

Gene specific primers were designed from the *RAD51* mRNA sequence above (GenBank Accession Nos. FJ518826) and using a published sequence for *M. edulis 18S rRNA* (L24489)(Table 2). *18S rRNA* has been validated previously as a suitable reference gene in mussels (Ciocan et al., 2011; Cubero-Leon et al., in press). Each primer pair was optimized and validated by the generation of standard serial dilution and melt curves on cDNA from *M. edulis* to ensure the formation of a single product with an appropriate melting temperature. The products of the optimized reactions were also analyzed by agarose gel electrophoresis to ensure that the size of the amplicon corresponded to the product of interest (121 bp, and 114 bp for *RAD51* and *18S* respectively) and sent for sequencing to confirm identity. The real-time PCRs consisted of 10 μl of qPCR FastStart Universal SYBR Green Master (ROX) (Roche Applied Science, U.K.), 4 μl of cDNA, 300 nM final concentration of *18S rRNA* forward and reverse primers and 600 nM final concentration of *RAD51* forward and reverse primers, and PCR-grade water to a total volume of 20 μl. The FastStart Universal SYBR Green Master contained 2×reaction buffer, 0.025 U μl^{-1} Taq Polymerase, 5 mM $MgCl_2$, dNTP mix (200 μM of each dNTP), ROX (passive reference dye) and SYBR Green.

All samples were analyzed in duplicate and with the following cycling parameters: 50 °C for 2 min, 95 °C 10 min followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 1 min and extension at 72 °C for 30 s. The products were slowly melted starting with 1 min at 95 °C followed by 30 s at 55 °C and 30 s at 95 °C. A negative control was set up along side each set of PCR reactions consisting of all components of the PCR reaction excluding the template DNA. The amplification was detected with a Mx3005P real-time PCR system (Stratagene, U.K.).

To evaluate PCR efficiency, precision and sensitivity, a standard curve was performed using a serial dilution of a positive template. Reaction efficiency for was 98.4% and 103.5% for *RAD51* and *18S rRNA* respectively. The efficiencies presented correlation coefficients of >0.99. All standards were amplified in triplicate and all experimental samples were assayed in duplicate. A relative quantitation method was chosen to analyze changes in mRNA expression of the target gene in the treatment group compared to a control sample. The results were normalized with a reference gene (*18S rRNA*). The method used to calculate the relative change values was the comparative ΔCt method using the formula $RQ = 2^{-\Delta Ct}$ where $\Delta Ct = Ct_{,RAD51} - Ct_{,18S}$ (Livak and Schmittgen, 2001).

Table 2
Oligonucleotide primer sequences for the amplification of *M. edulis RAD51* and *18S rRNA* mRNAs.

Primer sequence 5′–3′	
*18S*F	CAT TAG TCA AGA ACG AAA GTC AGA G
*18S*R	GCC TGC CGA GTC ATT GAA G
*RAD51*F	TGG CAT AGA GAC TGG GTC AA
*RAD51*R	CCT TCA CCT CCA CCC ATA TC

For statistical analysis (SPSS 15.0 for Windows), all data were tested for normality using the Shapiro–Wilk test. As all data were not normally distributed, non-parametric by Mann–Whitney *U* pair wise comparison tests were performed. $P < 0.05$ was considered significant.

3. Results

3.1. Partial RAD51 cDNA sequence characterization

A partial sequence for the *RAD51* cDNA (441 bp) was obtained by RT-PCR from *M. edulis* cDNA (GenBank Accession No. FJ518826). The BLAST algorithm confirmed the identity of the isolated fragment as a part of a putative *RAD51* gene. There was 87% similarity between the isolated fragment and *Dreissena polymorpha RAD51* (AF508221) and a range of similarity between 81 and 83% with *D. rerio*, *X. laevis* and human *RAD51* (NM_213206, NM_001087767 and D13804) respectively.

3.2. Radionuclide activities in mussel tissue and sediment samples

Specific activities of anthropogenic and naturally-occurring radionuclides in sediment and mussel tissue samples from RE were found to be similar to those reported previously (RIFE 14, 2009). For anthropogenic radionuclides, mussel tissue activities of 19 Bq kg^{-1} ^{239,240}Pu, 27 Bq kg^{-1} ^{90}Sr, 34 Bq kg^{-1} ^{241}Am and 5 Bq kg^{-1} ^{137}Cs were observed (Table 3). Sediment activities were *ca*. 83 Bq kg^{-1} ^{239,240}Pu, 35 Bq kg^{-1} ^{90}Sr, 170 Bq kg^{-1} ^{241}Am and 36 Bq kg^{-1} ^{137}Cs. With the exception of ^{210}Po and ^{90}Sr, activities were significantly lower at the reference site (BM). Gamma dose rates from sediment and rock substrates were higher at the RE site (0.09 ± 0.005 μGy h^{-1}) compared to the reference (BM) site (0.05 ± 0.01 μGy h^{-1}). Activities of naturally-occurring gamma-emitting radionuclides were also generally higher at the RE site (Table 3).

3.3. RAD51 mRNA expression and comet analysis in environmental reference and contaminated mussel samples

RAD51 mRNA expression in mussel gonad samples collected from the Ravenglass Estuary was significantly higher expressed compared with mussels sampled at the Brighton reference site (Fig. 1). Comet analysis showing as tail DNA% indicated the degree of DNA damage in mussel's haemocytes (Fig. 2). In the RE mussel samples, the DNA was significantly damaged (61.40 ± 2.09%) compared to mussel samples collected from both reference sites (12.69 ± 3.80% and 13.64 ± 1.99% for laboratory stabilized mussels and field collected mussels respectively) ($p < 0.05$). No significant difference in DNA damage between two reference mussel samples (Cornwall and Brighton) was observed.

4. Discussion

An integrated approach to investigate the potential biological impacts of ionizing radiation, including radiochemical analysis of environmental samples, molecular level analysis of RAD51 mRNA expression, and induction of DNA strand breaks (using comet assay), has been employed. To facilitate this approach the *RAD51* cDNA sequence was isolated from a commonly used bioindicator species, the mussel *M. edulis*, and a quantitative assay of its mRNA expression developed. The mussel RAD51 deduced amino acid sequence isolated is part of a conserved area, the putative ATP binding domains which contains the conserved Walker A and Walker B motifs, characteristic of a P-loop NTPase superfamily gene (Wiese et al., 2006). Conserved domains suggest that the function of this protein may be similarly conserved in the mussel.

Table 3
Radionuclide specific activities in sediment and mussel tissue samples. All data in Bqg^{-1} of dry sample. Uncertainties are propagated method uncertainties. Coverage factor $k = 2$ S.

Alpha and beta analysis				
Sample	^{210}Po	^{238}Pu	^{239,240}Pu	^{90}Sr
RE sediment	0.0049 +/− 0.0007	0.015 +/− 0.002	0.083 +/− 0.008	0.035 +/− 0.012
BM sediment	0.0031 +/− 0.0008	<0.0002	0.00017 +/− 0.00009	0.019 +/− 0.010
RE mussel	0.064 +/− 0.015	0.0037 +/− 0.0005	0.019 +/− 0.002	0.027 +/− 0.016
BM mussel	0.076 +/− 0.018	<0.0001	<0.0001	0.031 +/− 0.015

Gamma Spectrometry Artificial radionuclides										
Sample	^{241}Am	+/−	^{137}Cs	+/−	^{60}Co	+/−	^{155}Eu	+/−	^{65}Zn	+/−
RE sediment	0.1700	0.0100	0.036	0.020	<0.0008	–	<0.3	–	<0.002	–
BM sediment	<0.0009	–	<0.0006	–	<0.0009	–	<0.2	–	<0.002	–
RE mussel	0.0340	0.0030	0.0051	0.0016	<0.005	–	<0.6	–	<0.01	–
BM mussel	<0.004	–	<0.006	–	<0.009	–	<0.8	–	<0.02	–

Natural radionuclides																
Sample	^{228}Ac	+/−	^{40}K	+/−	^{210}Pb	+/−	^{212}Pb	+/−	^{214}Pb	+/−	^{226}Ra	+/−	^{234}Th	+/−	^{235}U	+/−
RE sediment	0.0072	0.0015	0.24	0.02	<0.02	–	0.0096	0.0008	0.0075	0.0007	< 0.02	–	<0.01	–	0.0008	0.0003
BM sediment	<0.003	–	0.016	0.008	<0.008	–	0.0028	0.0005	0.0034	0.0005	<0.01	–	0.0050	0.0018	0.0006	0.0003
RE mussel	<0.02	–	0.22	0.05	<0.02	–	0.0053	0.0019	<0.009	–	<0.04	–	<0.03	–	<0.008	–
BM mussel	<0.03	–	<0.2	–	<0.04	–	0.018	0.003	<0.02	–	<0.06	–	<0.05	–	<0.01	–

The levels of ionizing radiation were relatively low at the impacted site (i.e. RE). Focusing first on the gamma sources, ^{137}Cs values of 0.036 Bq g^{-1} dry weight sediment and 0.0051 Bq g^{-1} mussel tissue were detected at RE. The current levels are comparable with historic sampling values reported at this site (RIFE 14, 2009). The values are however higher than those reported along the United States coastline (with a maximum level reported of 0.0004 Bq g^{-1} mussel tissue) and the Mediterranean/Black Sea (with a maximum level of 0.0015 Bq g^{-1} mussel tissue) (Valette-Silver and Lauenstein, 1995; Thébault et al., 2008), yet significantly lower compared with *Spisula sachalinensis* bivalve tissue reported in 1991 from a sampling site in Japan (56 ± 3 Bq g^{-1} wet weight)(Yamada et al., 1999).

^{241}Am activities detected at RE in mussel tissue (0.034 Bq g^{-1} mussel tissue) are also higher compared with the reference site (<0.004 Bq g^{-1} mussel tissue), historic values at the same location (0.012 Bq g^{-1} mussel tissue) (RIFE 14, 2009), and relative to published data for other locations in the United States coastline (90 × 10^{-6} Bq g^{-1} mussel tissue) (Valette-Silver and Lauenstein, 1995). Activities of ^{241}Am in sediments similarly exceed those at the reference site. Of the naturally-occurring gamma-emitting radionuclides, highest activities are observed for ^{40}K at the RE site. These activities significantly exceed those at the BM site. Despite the slightly elevated activities observed for ^{241}Am and ^{137}Cs (and to some extent natural gamma-emitting radionuclides) at the RE site however, external gamma dose rates are not significantly elevated above those at the control site, and only slightly exceed those reported as background at other sites in the U.K. (RIFE 14, 2009).

The alpha and beta emitter activities detected are significantly higher in RE sediment and mussel tissue samples compared with reference sediments and tissue samples. Considering ^{210}Po, the levels detected in tissue from samples collected at the reference site BM (0.076 ± 0.015 Bq g^{-1} dry weight), were not significantly different from samples collected at the contaminated site, suggesting that ^{210}Po, at least, is not responsible for the comet damage or the elevated *RAD51* mRNA expression observed in the RE mussel tissues. Considering the alpha and beta emitters present, while similar activities of ^{210}Po and ^{90}Sr are observed at each site, ^{238,239,240}Pu, and ^{241}Am (as well as the natural radionuclide ^{40}K – see above) activities in mussel tissue at RE are all significantly

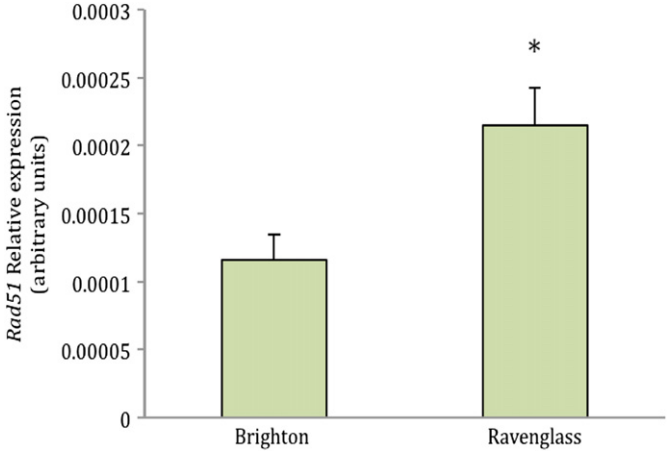

Fig. 1. *RAD51* mRNA expression in gonad of mussels sampled from Ravenglass Estuary and Brighton. The figure shows relative *RAD51* gene expression to *18S rRNA* reference gene expression. $n = 9$. Mean data are plotted ± SEM. * indicates significant difference ($p < 0.05$).

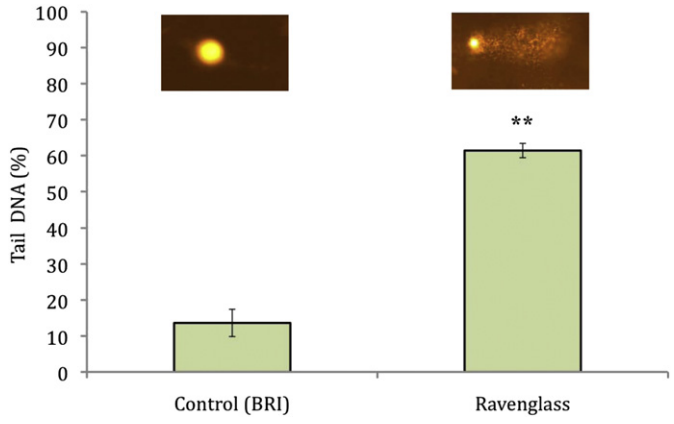

Fig. 2. Induction of DNA strand break (represented as % Tail DNA) in *Mytilus sp*. haemocytes collected from a radionuclide contaminated site (Ravenglass) and a reference site (Brighton Marina). $n = 9$. ** indicates significant increase of % Tail DNA in contaminated groups compared with reference group ($p < 0.05$). Pictures show comet assay image at different conditions.

higher compared with the activities detected in BM mussel tissue samples, as well as levels detected along the United States coastline (Valette-Silver and Lauenstein, 1995). ^{99}Tc was not measured in this study, yet it has been identified previously in RE shellfish tissues at levels of 0.18 Bq g^{-1} and 0.172 ± 0.205 Bq g^{-1} wet weight mussel and crab tissue samples respectively (RIFE 14, 2009; Swift and Nicholson, 2001), so may also contribute to the total beta dose at the RE sample site.

The apparent elevated chronic alpha and beta exposure is one possible cause of the increased DNA damage detected using comet analysis and the elevated *RAD51* mRNA expression at the RE sampling site compared with the reference site. Examining the general DNA damage first, a significant increase in DNA damage measured using the comet assay was observed in the RE mussel haemocytes compared to mussels collected from the reference site (Fig. 2). This finding is consistent with a previous study in which *M. edulis* were experimentally exposed to tritiated water with a chronic low dose of 12 μGy h^{-1} for 96 h (Jha et al., 2005). It is in contrast, however, to a second study in which brown mussel (*Perna perna*) were sampled from a site of chronic ionizing radiation contamination burden (measured as ^{210}Po), twice that detected at the RE site, and an absence of DNA damage reported (Godoy et al., 2008). Unlike *RAD51* expression, as mentioned earlier, DNA SSBs measured using comet assay is not specific to ionizing radiation and can be induced by other classes of common environmental contaminants such as heavy metals and PAHs (Banni et al., 2010; Di et al., 2011; Millward et al., 2012). In the catchment surrounding RE, there has been a long tradition of heavy metal ore mining (Mayes et al., 2009), but levels of such contaminants in the area are not readily available in the literature. The East Irish Sea and surrounding coastline is also an area of high industrial and shipping activity, where total hydrocarbon concentrations in the range of 3–12 μg l^{-1} have previously been reported (Law, 1981). In an experimentally-controlled laboratory exposure, a single (acute) PAH concentration of 5.6 μg l^{-1} induces significant levels of DNA strand breaks using the comet assay (Di et al., 2011). Others report that 19 μgl^{-1} benzo(a)pyrene induces significant DNA strand damage in *M. galloprovincialis* using this assay (Banni et al., 2010). The increase in DNA damage measured using the comet assay may thus be due to a combination of environmental contaminants including radionuclides, metals and organics present at the RE sampling site.

Activities of alpha, beta and gamma-emitting radionuclides measured in sediment and mussel tissue at the RE site translate into external and internal dose rates that are low, at *ca.* 0.61 μGy h^{-1} (estimated following Blaylock et al. (1993)), which is significantly lower than the generic (all species) "no effect" dose rate of 10 μGy h^{-1} proposed by Andersson et al. (2009). Yet, at this low level of contamination a significant increase in *RAD51* mRNA expression levels was observed in mussel tissue samples compared with the samples from the reference site. RAD51 is an important part of the homologous recombination DNA repair pathway that is involved specifically in response to DSBs (for review: Masson and West, 2001; Thacker, 2005), indicative of physically-, rather than chemically-, induced SSBs in DNA. In particular, high LET radiations (e.g. alpha particles) are very efficient in inducing DSBs (Natarajan et al., 1993) and it is therefore not surprising to have elevated levels of *RAD51* mRNA expression in the mussel tissue in our study. Given that RAD51 is specific to high LET ionizing radiation-induced DSBs, an increase in the *RAD51* mRNA expression, following chronic environmental exposure, indicates that the cell's DNA repair mechanism for DSBs has been triggered, even at relatively low and chronic (environmental) exposure to ionizing radiation.

In the environment, where physical and chemical contaminants occur in combination, our results specifically indicate an induction of DSBs likely induced by ionizing radiation with high LET values (Natarajan et al., 1993). Presence of low LET radiation qualities and chemical contaminants, which predominantly induce SSBs, could also contribute to generation of DSBs, in theory, but only if they occur spatially very close to each other in the genome (Jackson and Bartek, 2009). Induction of *RAD51* mRNA expression therefore could result from both high and low LET ionising radiations with potential, but limited, contributions from chemical contaminants.

One interpretation of the increased *RAD51* mRNA expression, at low dose of ionizing radiation such as those detected at the RE site, is the fact that expression of genes is the first step towards response to any environmental stresses. A large number of studies in aquatic organisms have investigated enhanced gene expression following exposure to different contaminants (Ciocan and Rotchell, 2004; Ciocan et al., 2010, 2011; Nogueira et al., 2010; Di et al., 2011). Whilst there have been a large number of studies related to key genes involved in genotoxic responses (e.g. *p53, ras*) in aquatic organisms, the application of *RAD51* gene as a specific marker for DSBs is novel. Our integrated study therefore contributes to the limited amount of information pertaining to expression of a key gene and DNA damage in mussels following exposure to radioactivity in the natural environment.

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