

Assessing the impact of ionising radiation on bivalves

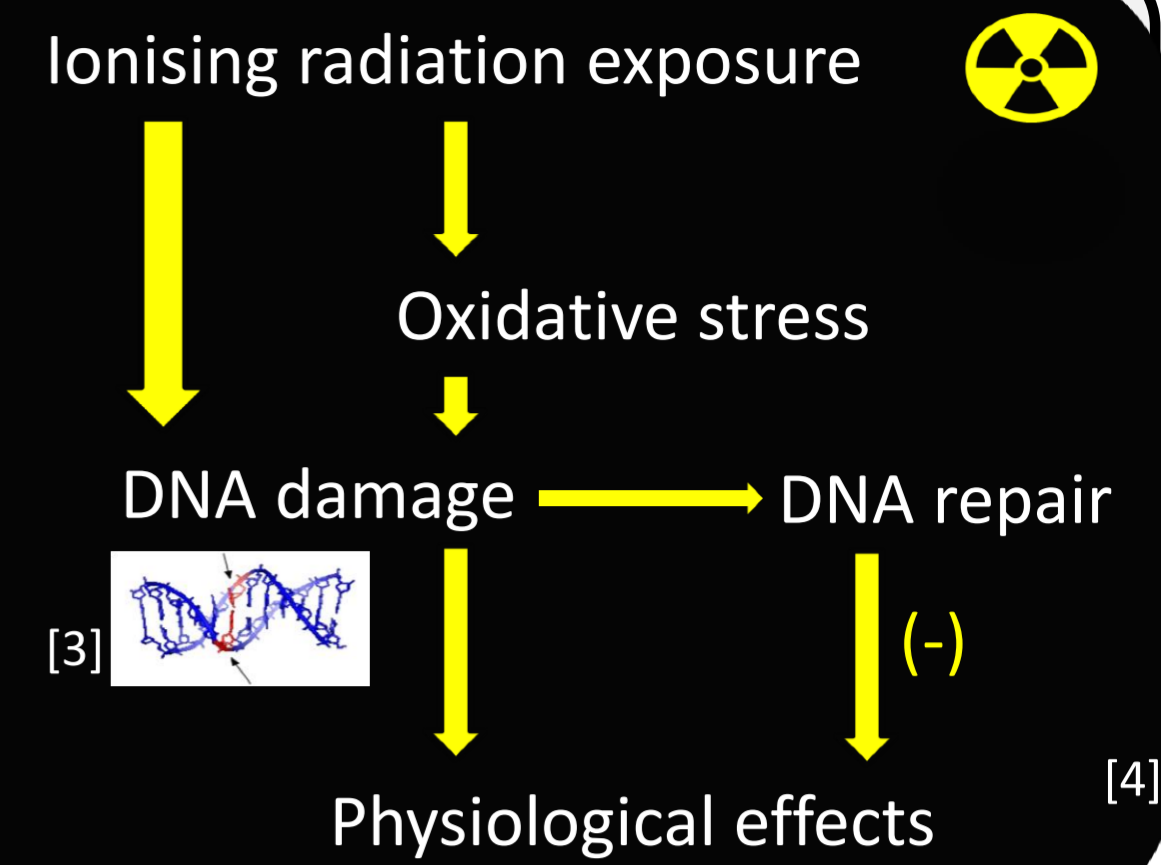


Emily Vernon. Plymouth University



BACKGROUND

- ❖ The aquatic environment is the main recipient of anthropogenic contaminants, including radionuclides
- ❖ Growing environmental and human health concern over the presence of radionuclides
- ❖ Aquatic organisms play an important role in ecosystem functioning, bivalve molluscs particularly important for several reasons **(a)** Economic value **(b)** Ecological role: Keystone species **(c)** Environmental biomarker – useful for environmental bio-monitoring²
- ❖ Genetic information (DNA) is an important target for the action of ionising radiation



AIMS

- ❖ To determine a range of biological responses in bivalve molluscs following exposure to a range of radionuclides of differing emission characteristics, either alone or in combination with additional environmental stressors
- ❖ Establish potential links between DNA damage and higher levels of biological organisation (i.e tissue, individual, germ cell and transgenerational effects)

OBJECTIVES

- (1) Confirm the species to be used in the experiments using molecular techniques**
- (2) Quantify the induction of DNA damage and repair in different tissues using a range of techniques**
- (3) Determine the transcriptional expression of genes involved in DNA damage response (DDR) and stress response**
- (4) Determine histological and physiological effects. Establish potential links with DNA damage and molecular response**
- (5) Determine DNA damage in germ cells (i.e sperm) and correlate it with fertilisation success potential**

Clearance rate (CR)

An estimation of the rate at which a bivalve molluscs filter water
Useful as a measurement of physiological effects – CR affected by environmental stressors⁵

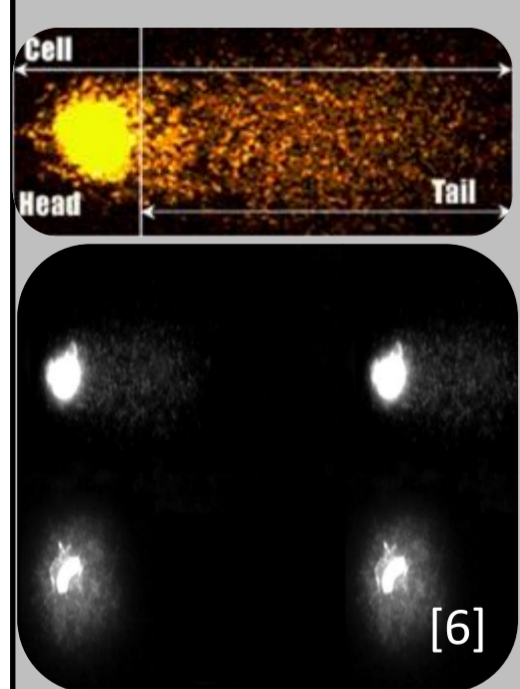


DNA strand breaks in germ cells

DNA damage at the development stages (germ cells i.e. sperm) can have potential long-term consequences for population success (i.e fertilisation success, developmental abnormalities)¹²

DNA damage and repair

The **comet assay** (single-cell gel electrophoresis) - used to detect DNA strand breaks and repair in cells
Comet tail intensity corresponds to the amount of DNA damage



Immunocytochemical assays:

53BP1 induction⁷ and γ -H2AX (key factors in the repair process of DNA)⁸



APPROACH AND METHODOLOGY

Histology

Light and electro-microscopic approach to study histological sections of mussel tissues

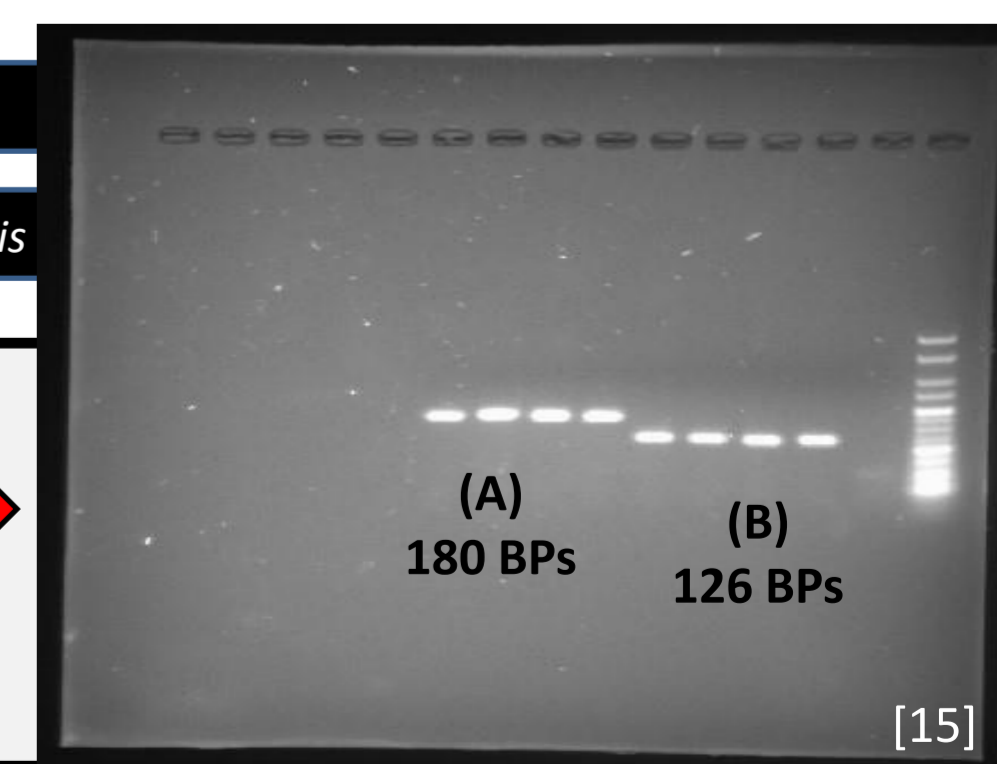


Light micrograph sections through male and female gonads (*M. edulis*)¹⁰

Transcriptional expression of genes

Investigation into the role of genes encoding proteins known to be involved in DDR and DNA repair. Real-time PCR to quantify the expression levels of genes (inc. *p53*, *RAD* etc. and antioxidants e.g. superoxide dismutase, metallothionein etc.)¹¹

A) *M. edulis*
B) *M. Galloprovincialis*



CURRENT RESEARCH WORK

1. Identification and confirmation of mussel species using the Glu 5 gene as marker (PCR)¹⁴
2. Optimisation/validation of DDR in different tissues using comet assay and immunocytochemical assays

Contact: emily.vernon@plymouth.ac.uk. PhD supervisors: Professor Awadhesh Jha, Professor Jim Smith, Dr Alex Ford.

References: (1) I-aquaculture.blogspot.com (2) Lonsdale *et al* (2009) *Aquatic Biology* 6(1-3), 263–279 (3) www.scientificamerican.com (4) Won *et al* (2014) *Env Sci Pol Res*. (5) Petersen *et al* (2004) *Marine Ecology Progress Series* 267, 187-194 (6) www.libyanjournalofmedicine.net & jhc.sagepub.com (7) Panier *et al* (2013) *Nature Reviews Molecular Cell Biology* 15, 7–18 (8) Srivastava *et al* (2009) *Mutation research/Reviews in mutation research* 681, 180-188 (9) Human linfoid tissue labelling 53BP1 (www.abcam.com) (10) Sheir, *et al* (2013) *Arch Environ Contam Toxicol* 64, 701–716 (11) Farcy *et al* (2007) *Science of the Total Environment* 374, 328–341 (12) Lewis *et al* (2008), *Environ Sci Technol* 43, 928-933 (13) www.seagrant.umaine.edu (14) Inoue *et al* (1995), *The Biological Bulletin* 189(3), 370–375 (15) Mussel identification. Dr L Dallas. 2014.