Report No. 143

epaResearch
Climate - Water - Sustainability
*Identifying pressures • Informing policy • Developing solutions*

# Pharmaceuticals in the Irish Aquatic Environment

Comhshaol, Pobal agus Rialtas Áitiúil
Environment, Community and Local Government

www.epa.ie

**EPA Research Programme 2014–2020**

# Pharmaceuticals in the Irish Aquatic Environment: The Assessment and Potential Human Impact of Exposure to Pharmaceuticals on Marine and Freshwater Bivalves

## EPA Research Report 143

Prepared for the Environmental Protection Agency

by

Irish Centre for Environmental Toxicology, GMIT

**Authors:**

**Brian Quinn, Gillian McEneff and Wiebke Schmidt**

**ENVIRONMENTAL PROTECTION AGENCY**

An Ghníomhaireacht um Chaomhnú Comhshaoil
PO Box 3000, Johnstown Castle, Co. Wexford, Ireland

Telephone: +353 53 916 0600 Fax: +353 53 916 0699
Email: info@epa.ie Website: www.epa.ie

## DISCLAIMER



The EPA Research Programme addresses the need for research in Ireland to inform policymakers and other stakeholders on a range of questions in relation to environmental protection. These reports are intended as contributions to the necessary debate on the protection of the environment.

**EPA Research Programme 2014–2020**

Published by the Environmental Protection Agenc y, Ireland

ISBN: 978-1-84095-580-4

Price: Free **Online version**

# ACKNOWLEDGEMENTS

This report is published as part of the EPA Research Programme. The Research Programme is administered on behalf of the Department of the Environment, Community and Local Government by the Environmental Protection Agency which has the statutory function of co-ordinating and promoting Irish environmental research.

The authors are grateful to all those who contributed at various levels to the success of this project (for a full list see Appendix 2). They gratefully acknowledge the support, encouragement and advice from the members of the steering committee: Dr Shane Colgan (Environmental Protection Agency), Prof. Martin Cormican (National University of Ireland, Galway), Prof. David Sheehan (University College Cork), Prof. Susana Cristobal (Linköping University), Prof. Brett Paull (Dublin City University), Prof. Jim Wilson and Dr Michelle Gilltrap (Trinity College Dublin) and Dr Rick Officer (Galway-Mayo Institute of Technology). Thanks are also offered to Prof. Francois Gagné of Environment Canada for his advice during the project. Prof. David Sheehan and Dr Louis-Charles Rainville (University College Cork) are thanked for hosting the PhD student Wiebke Schmidt in their laboratory. The authors are grateful to the staff at both the sewage treatment plants that were sampled and at ESB Poolbeg for allowing them access to their sites. Particular thanks go to Dr Damian Griffin (University College Hospital, Galway) and to Vincent Foley and Olive McGann (Cruinn Diagnostics) for their support and technical help with the environmental diagnostics work. They are very grateful for various significant contributions made by Robert Hernan, Kathleen O'Rourke and Liam Curran in the Shannon Aquatic Toxicity Laboratory. Many thanks are also due to Dr Iarlaith Connellan (Redbank Hatchery) and Mark Jessopp (University College Cork) for the provision of samples. Final thanks to Dr Brendan McHugh, Dr Jenny Ronan (Marine Institute) and Prof. Jim Wilson and Dr Michelle Giltrap (Trinity College Dublin) who contributed to the in situ exposures.

# Details of Project Partners

**Brian Quinn* (Principal Investigator)**
Institute of Biomedical & Environmental Health Research
University of the West of Scotland
School of Science
Paisley PA1 2BE
Scotland
Email: brian.quinn@uws.ac.uk

**Gillian McEneff**, Leon Barron**
Department of Pharmacy and Forensic Science
Analytical & Environmental Science Division
King's College London
Franklin Wilkins Building
150 Stamford Street
London SE1 9NH
United Kingdom
Email: gillian.mceneff@kcl.ac.uk

**Wiebke Schmidt**
European Centre for Environment and Human Health (ECEHH)
University of Exeter Medical School
Knowledge Spa
Royal Cornwall Hospital
Truro TR1 3HD
Cornwall
United Kingdom
Email: schmidt.wiebke@gmail.com

**Vincent Foley, Olive McGann**
Cruinn Diagnostics Ltd
5b/6b Hume Centre
Park West Industrial Estate
Nangor Road
Dublin 12
Ireland

**Brett Paull**
Australian Centre for Research on Separation Science (ACROSS)
School of Chemistry
University of Tasmania
Hobart
Tasmania
Australia

**Brian Kelleher**
Irish Separation Science Cluster (ISSC)
Dublin City University
Dublin 9
Ireland

***Previous address:***
Irish Centre for Environmental Toxicology
Galway–Mayo Institute of Technology
Dublin Road
Galway
Ireland

****Previous address:***
Irish Separation Science Cluster (ISSC)
Dublin City University
Dublin 9
Ireland
*and*
Irish Centre for Environmental Toxicology
Galway–Mayo Institute of Technology
Dublin Road
Galway
Ireland

# Table of Contents

# Executive Summary

## 1 Background

In March 2008, the Irish Environmental Protection Agency (EPA) funded a 5-year research project *The Assessment and Potential Human Impact of Exposure to Environmental Contaminants on Marine and Freshwater Bivalves* (2007-DRP-3-S5) under the Science, Technology, Research and Innovation for the Environment (STRIVE) Developing Environmental Research Potential (DERP) scheme. This project specifically investigated pharmaceuticals as environmental contaminants of emerging concern and arose out of the lack of research on their presence in and potential impact on the Irish aquatic environment. Internationally, pharmaceuticals have recently become the object of considerable attention and concern due to their possible toxicity and potential to cause adverse effects in aquatic organisms and the potential for direct human exposure via ingestion of contaminated seafood.

To assess the impact of a pollutant on the environment, both chemical (to identify the compounds and calculate their concentration) and biological analyses (to investigate their toxicity potential) must be undertaken. This project is therefore divided into two broad areas – chemistry and biology. The overall aim of this project was to combine chemical and biological analyses in an integrated assessment of the extent and the effects of pharmaceutical pollution in the Irish aquatic environment, with the ultimate aim of developing a simple bioassay for the fast, reliable identification and toxicological assessment of pharmaceuticals in environmental samples.

## 2 Objectives

- To develop a validated protocol for the identification and quantification of pharmaceuticals in various environmental matrices (effluent, marine water and biological tissue);
- To measure chosen pharmaceuticals in municipal effluent, receiving marine waters and marine biota;
- To assess the potential for human exposure via ingestion of contaminated seafood;
- To investigate the toxic potential of pharmaceuticals to aquatic organisms, focusing on sublethal, chronic effects; and
- To adapt human diagnostic techniques for environmental monitoring (environmental diagnostics).

## 3 Key Outputs

Although a previous EPA-funded study had reported the presence of pharmaceuticals in municipal effluents[1], the current study is the only report on the spatial occurrence and relative distribution of pharmaceutical residues in the Irish marine environment and their bioaccumulation potential.

The information gathered using the developed analytical techniques has enhanced Ireland's capacity towards the integrated monitoring of contaminants in the marine environment and these findings will contribute to future pharmaceutical fate studies and evaluations of the human risks posed by these emerging environmental pollutants.

The scope of the biological assessment presented in this report has provided vital knowledge of the potential toxic effects of pharmaceuticals on non-target aquatic organisms. The information gathered using biomarker techniques has also enhanced Ireland's capacity towards integrated pollution monitoring, providing valuable baseline data on biomarker responses.

1. Lacey, C., McMahon, G., Bones, J., Barron, L., Morrissey, A. and Tobin, J.M. 2008. An LC-MS method for the determination of pharmaceutical compounds in wastewater treatment plant influent and effluent samples. *Talanta* **75:** 1089–1097.

Although not yet validated, the use of human diagnostic techniques for environmental assessment has shown promising results, with the ability of these technologies to cross-react and measure the appropriate end points in various aquatic species, offering a potentially useful tool for environmental monitoring.

# 1 Introduction

## 1.1 Background

Pharmaceutical drugs are described as contaminants of emerging concern (CECs) in the aquatic environment. The amount of pharmaceutical production, consumption and ultimately discharge into the aquatic environment is steadily increasing (EEA, 2010). Although much research has recently been undertaken on the chemical analysis of pharmaceuticals in fresh waters, little is known about their fate in the marine environment and their toxicity potential. In Ireland, the Irish Medicines Board[1] (IMB) has licensed more than 6,000 medicines for human use and more than 1,000 for veterinary use (Barron et al., 2008). Ireland is a leading location for the pharmaceutical industry, housing nine of the 10 largest pharmaceutical companies in the world. The Irish market for pharmaceutical products in 2010 was valued at just under €2.3 billion by the Irish Pharmaceutical Healthcare Association (IPHA), indicating a high level of pharmaceutical consumption in Ireland (IPHA, 2012). Despite the growing concern and volume of publications on pharmaceuticals as environmental pollutants, no study had previously investigated the occurrence or potential impact of these compounds in Irish waters.

The European Union (EU) Water Framework Directive's (WFD) list of priority substances lists 33 priority pollutants and eight other pollutants that must be regulated and monitored in all European wastewaters. Following recent findings, the European Commission (EC) has revised this list and included the regulation of 12 new substances and the monitoring of three pharmaceutical compounds – the anti-inflammatory drug diclofenac and the hormones 17α-ethinylestradiol and 17β-estradiol (EU, 2013). These compounds have been added to a 'watch list' of compounds which are not subject to EU standards but are instead closely monitored in EU surface waters for possible future addition to the priority list. The recently implemented Marine Strategy Framework Directive (MSFD) extends EU water legislation to the marine environment. Thereby it follows the approach of the WFD to achieve and ensure a 'Good Environmental Status' (GES) by 2020. Both Directives (WFD and MSFD) are linked and will provide protection and management of Europe's freshwater, coastal and marine waters. These regulatory Directives provide the framework for the chemical analysis (of both municipal and the receiving marine environment) and environmental toxicity testing of pharmaceuticals that was undertaken in the current project.

Pharmaceuticals primarily enter the aquatic environment via treated municipal wastewater released by wastewater treatment plants (WWTPs). In Ireland, these are primarily located along the coast, potentially impacting on the marine and estuarine environment. Human actions, termed as 'involuntary' (pharmaceutical excretion through the body or washing of topical medicines down the drain) and 'purposeful' (disposal of unused or out of date medicines) are primarily responsible for the release of pharmaceuticals into the environment (Daughton, 2007). Human pharmaceuticals are excreted into the sewage system as a mixture of the parent compound and its metabolites. A previous Environmental Protection Agency (EPA) study identified 15 pharmaceutical compounds in influent and effluent samples from three Irish WWTPs in the high (ng/l) to low (µg/l) range (Lacey et al., 2008). Medicines disposed of inappropriately into refuse waste enter landfill sites and can leach into the soil. Agricultural medicines administered to farmed animals are also a source of pharmaceutical pollution. In addition, sludge from WWTPs is used as a soil fertiliser and may also be a source of pharmaceutical contamination in the environment (Barron et al., 2009). Other sources of pharmaceutical pollution of the aquatic environment include industrial spills and aquaculture.

Pharmaceuticals are designed to have a specific structure, mode of action and biological effect which determine their function and therapeutic class. They

1. Now the Health Products Regulatory Authority (HPRA) since July 2014.

can be classified into numerous therapeutic classes, including anti-inflammatories, antibiotics, antipsychotics, antihypertensives, antidiabetics, antihistamines, lipid regulators, anticonvulsants, β-blockers, stimulants, sex hormones, statins and many more. Acute and chronic standard toxicity tests are currently used to evaluate the potential environmental risk of pharmaceuticals on non-target organisms. Acute toxicity is generally measured over a shorter exposure period (e.g. 24 h), often, but not exclusively, with lethality as the end point, whereas chronic toxicity describes adverse effects (often sublethal, including, for example, physiological or biochemical changes) observed following an extended exposure period. The current list of pharmaceutical compounds deemed harmful to the aquatic environment is compiled based on acute toxicity tests. Of the 94 publications from 1996 to 2009 reviewed by Santos et al. (2010), almost 70% reported acute toxicity data. However, as pharmaceuticals are found in the environment at high (ng/l) to low (µg/l) concentrations, short-term acute toxicity tests of high concentrations may not be relevant to assess their toxicity. Longer-term chronic exposures to more environmentally relevant concentrations are preferred. At the present time, ecotoxicological data are available either in peer-reviewed literature or ecotoxicological databases for less than 10% of the currently prescribed pharmaceuticals (Brausch et al., 2012). Given the continuous release of pharmaceutical products into the environment, short-term acute toxicity tests are only relevant when accidental discharge of a drug occurs (Santos et al., 2010). In addition, the majority of research performed on testing potential adverse effects of pharmaceuticals has been based on individual-substance exposures in laboratory waters, rather than on effluent matrices or pharmaceutical mixtures (Brausch et al., 2012). Research in this area is still in its early stages, although this knowledge is steadily increasing.

In Ireland, WWTPs are primarily located along the coast, allowing the potential for pharmaceuticals to impact on estuarine and marine environments. Reports concerning the quantitative analysis of pharmaceuticals in marine ecosystems are somewhat limited. It is necessary to determine pharmaceutical fate and assess any potential risk of exposure to aquatic species and ultimately to seafood consumers, as the knowledge of the potential toxicological effects of these novel contaminants in non-target organisms remains incomplete. Bivalve mussels, such as the marine blue mussels (*Mytilus* spp.) and freshwater zebra mussel (*Dreissena polymorpha*) are commonly used in ecotoxicology and have been extensively used in various monitoring programmes, such as the Mussel Watch Programme (Kimbrough et al., 2008). Due to their wide distribution, sentinel, long life cycle and filter-feeding behaviour they are a good bioindicator species for pollution.

This report is a summary of the 5-year EPA-funded Developing Environmental Research Potential (DERP) project *The Assessment and Potential Human Impact of Exposure to Environmental Contaminants on Marine and Freshwater Bivalves*. The extensive report containing the full results of the project is summarised in Appendix 1 and can be found on the EPA SAFER website (http://erc.epa.ie/safer/reports).

## 1.2 Project Description

To assess the impact of a pollutant on the environment both chemical (to identify the compounds and calculate their concentration) and biological analyses (to investigate their toxicity potential) must be undertaken. This project is divided into these two main parts. The chemical analysis (effluent, receiving waters and biota) was undertaken in the Irish Separation Science Cluster (ISSC) in Dublin City University (DCU), with the biological analysis undertaken in the Irish Centre for Environmental Toxicology in the Galway–Mayo Institute of Technology (GMIT). Biological exposures were undertaken at in vitro, in vivo and in situ levels. An overview of the entire project is presented in Fig. 1.1.

## 1.3 Project Objectives

The overall objective of this project was to combine chemical and biological analysis in an attempt to assess, using an integrated approach, the fate and potential effects of pharmaceutical pollution in the Irish aquatic environment, with the ultimate aim of developing a simple bioassay for fast, reliable identification and toxicological assessment of pharmaceuticals in environmental samples. The major objectives of the project were to:

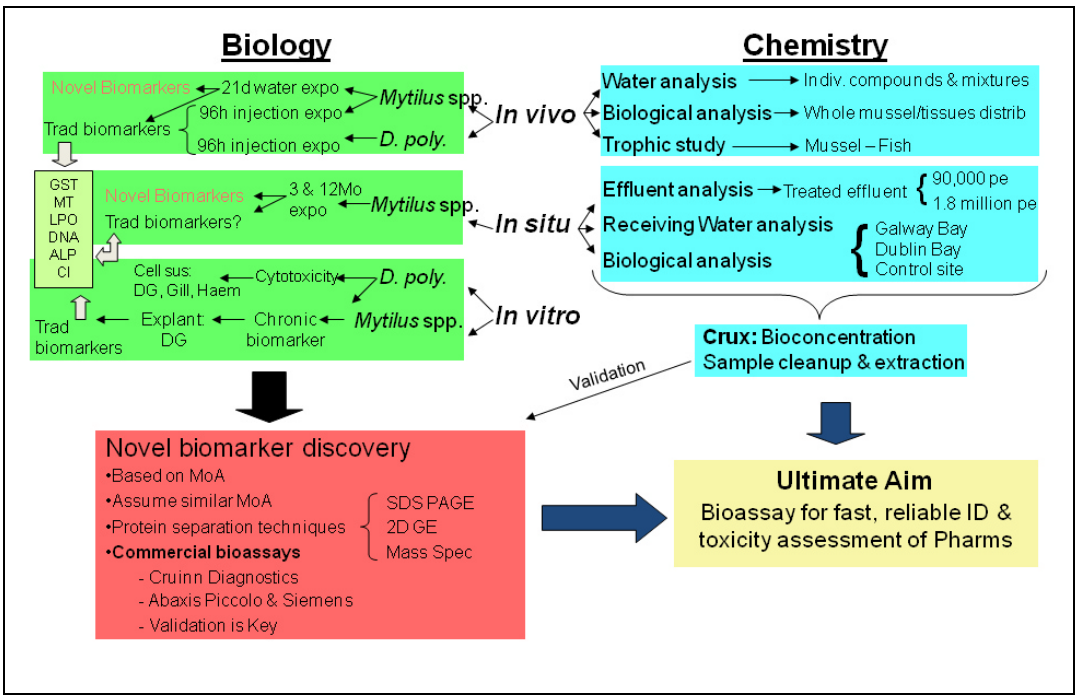



**Figure 1.1. Overview of the objectives and ultimate aim of this Developing Environmental Research Potential (DERP) project, including both chemical and biological analyses. *D. poly.*, *Dreissena polymorpha*; Expo, exposure; Mo, Month; DG, digestive gland; Haem, haemolymph; GST, glutathione-S-transferase; MT, metallothionein; DNA, DNA damage; LPO, lipid peroxidation; ALP, alkali-labile phosphates; CI, condition index; MoA, mode of action; pe, population equivalent.**

- Develop and validate a method for the analytical measurement of selected pharmaceutical compounds in effluent, marine waters and biota;
- Assess the potential of these selected compounds to bioaccumulate in the mussels and their availability for human consumption;
- Use emerging techniques in proteomics for the development of novel biomarkers to indicate pollution by pharmaceuticals based on a specific mode of action. Confirm this response with traditional biomarkers;
- Develop a small-scale toxicity test kit, based on the adaptation of human medical techniques (environmental diagnostics); and
- Develop and test in vitro techniques to investigate both cytoxicity and biomarker expression to aid a real in vitro/in vivo comparison.

# 2 Research Approach, Actions and Results

## 2.1 Chemical Analysis of Pharmaceuticals in the Irish Aquatic Environment (Water and Biota) and the Potential for Human Exposure

Part of the chemical analysis of this project formed the optimisation and validation of analytical methods for the quantification of five pharmaceutical residues in wastewater effluent, receiving marine waters and marine mussels (*Mytilus* spp.). Selected pharmaceuticals included two non-steroidal anti-inflammatory drugs (diclofenac and mefenamic acid), an antibiotic (trimethoprim), an antiepileptic (carbamazepine) and a lipid regulator (gemfibrozil). For aqueous samples, pharmaceutical residues were determined using solid phase extraction (SPE) and liquid chromatography–tandem mass spectrometry (LC-MS/MS). The extraction of pharmaceuticals from mussel tissues used an additional pressurised liquid extraction (PLE) step prior to SPE and LC-MS/MS. Limits of quantification (LOQs) of ≤225 ng/l were achieved in wastewater effluent, ≤38 µg/l in marine surface water and ≤29 ng/g in marine mussels. Method linearity was achieved for pharmaceuticals in each matrix with correlation coefficients of $R^2 \geq 0.976$. All five selected pharmaceuticals were quantified in wastewater effluent and marine surface waters (Table 2.1). This work demonstrated the susceptibility of the *Mytilus* spp. to pharmaceutical exposure following the detection of pharmaceutical residues in the tissues of this mussel species at measurable concentrations (McEneff et al., 2014).

Furthermore for the first time, the spatial occurrence of five targeted pharmaceuticals in the aquatic environment was monitored over a 12-month period (McEneff et al., 2014). Analytical techniques such as PLE, SPE and LC-MS/MS were combined, optimised and applied to wastewater effluent, marine surface water and *Mytilus* spp. samples, collected from two impacted sites and a control site on the Irish coastline. The presence of all five targeted pharmaceuticals was confirmed in the low (µg/l) in effluent and in the high (ng/l) ranges in exposed marine surface water (Table 2.1). Residues of carbamazepine measured highest in exposed marine surface water at concentrations up to 1.41 µg/l. Three of the five detected pharmaceuticals in marine surface waters were also found to occur in exposed *Mytilus* spp., with residues of trimethoprim measuring at concentrations up to 9.22 ng/g dry weight (DW). This study has confirmed the uptake of pharmaceuticals in marine bivalves at measurable quantities and also highlights the inability of mussels to act as reliable bioindicators of pollution for the selected pharmaceuticals due to temporal variations observed in the data. These findings will contribute to pharmaceutical fate studies and aid in the assessment of ecological and health risks posed by these contaminants in the natural aquatic environment.

**Table 2.1. Range of pharmaceutical concentrations measured in effluent and sea water at Galway Bay and Dublin Bay for the 3 and 12-month sampling periods, respectively.**

| | Effluent (µg/l) | Sea water (µg/l) |
|---|---|---|
| **Carbamazepine** | | |
| **Galway Bay** | 0.2–3.5 | 0.004–0.6 |
| **Dublin Bay** | 0.3–3.9 | 0.2–2.0 |
| **Mefenamic acid** | | |
| **Galway Bay** | 0.3–3.8 | <0.03–1.1 |
| **Dublin Bay** | <0.22–2.7 | <0.03–0.8 |
| **Diclofenac** | | |
| **Galway Bay** | 0.3–2.8 | 0.1–0.7 |
| **Dublin Bay** | 0.2–2.3 | <0.02–0.6 |
| **Trimethoprim** | | |
| **Galway Bay** | 0.3–1.3 | 0.4–1.0 |
| **Dublin Bay** | 0.1–1.6 | <0.006–0.9 |
| **Gemfibrozil** | | |
| **Galway Bay** | <0.04–1.7 | <0.04–1.0 |
| **Dublin Bay** | <0.04–1.0 | <0.04–0.3 |

The effect of cooking (by steaming) on pharmaceutical residues present in marine mussels (*Mytilus* spp.) was investigated to determine any potential difference in human exposure risk (McEneff et al., 2013). A preliminary in vivo exposure experiment was set up in the laboratory in which mussels were exposed either directly by injection (10 ng per mussel) or daily through spiked artificial sea water (ASW) over 96 h. The optimised SPE and LC-MS/MS methods for marine surface water were applied to ASW samples. LOQs of ≤46 ng/l were achieved for extracted cooking water and ASW, ≤64 µg/l for ASW in exposure tanks, and ≤29 ng/g for mussel tissue. Method linearities were achieved for pharmaceuticals in each matrix with correlation coefficients of $R^2 \geq 0.977$. A selection of exposed mussels was steamed and analysed using the optimised method to observe any effect on detectable concentrations of parent pharmaceuticals present. Domestic cooking by steaming resulted in an overall increase in pharmaceutical residues in the contaminated mussel tissue and cooking water. Diclofenac, gemfibrozil and mefenamic acid residues in the mussel tissue increased by more than a factor of 20 and in the case of mefenamic acid, concentrations increased from 1.6 µg/g in the raw mussel to 89.6 µg/g after cooking (Fig. 2.1). Acidic pharmaceuticals undergo high rates of biotransformation in the mussel due to large increases in glutathione-S-transferase (GST) enzyme. These glucuronides are generally stable but it is hypothesised that their reconversion to the parent compound occurred following thermal treatment, resulting in the increases found compared with the uncooked mussels (McEneff et al., 2013). The basic compound, trimethoprim, was the only pharmaceutical to undergo a reduction of 23%, due to its polar nature. Carbamazepine, a neutral compound, underwent the least change in concentration, with an increase of 12% after cooking. The potential risk of pharmaceutical exposure to humans through the food chain is low when pharmaceuticals are present in foods at environmentally relevant concentrations. However, this work has highlighted the possibility for pharmaceutical residues in food, particularly acidic compounds, to increase in concentration after thermal treatment and potentially pose a risk to human health via ingestion of farmed fish or animals regularly administered medication at high doses.

The potential for these pharmaceuticals to bioaccumulate in aquatic species, such as fish, via trophic-level transfer was also investigated. An in vivo experiment was carried out in a flow-through system in which juvenile rainbow trout were fed marine mussels (*Mytilus* spp.) collected from a highly polluted site exposed to wastewater effluent for a period of 28 days. The pharmaceutical extraction method applied to marine mussels was further optimised for fish liver samples with LOQs of ≤53 ng/g and correlation

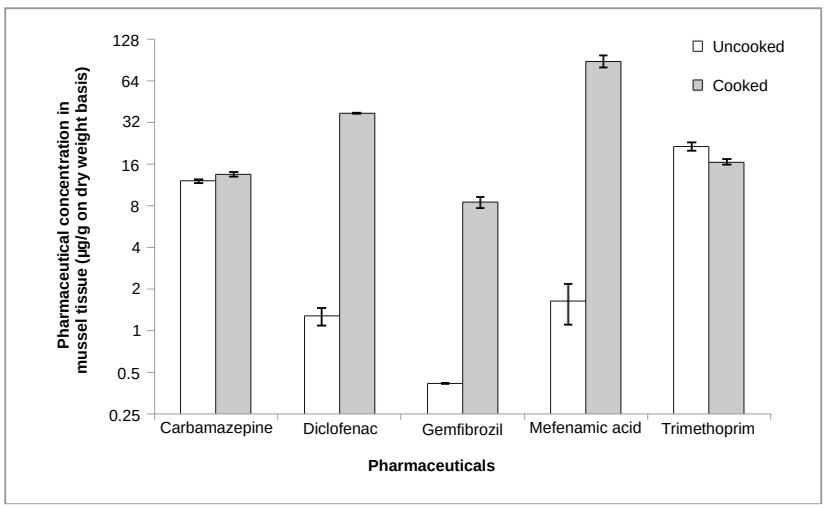



**Figure 2.1. Pharmaceutical concentrations (µg/g dry weight) detected in mussel tissue extracts before (n = 2) and after cooking (n = 2). Water-exposed mussels sampled at $t_{96h}$ were steamed and compared with uncooked mussels also sampled at $t_{96h}$.**

coefficients of $R^2 \geq 0.988$ achieved. Trimethoprim was the only pharmaceutical to be quantified in the wild *Mytilus* spp., at a concentration of 6.68 ng/g DW. After feeding, none of the selected pharmaceuticals was detected in the fish liver samples collected from the exposed rainbow trout. Hence, the potential for pharmaceuticals, in particular trimethoprim, to biomagnify via trophic-level transfer is low; however, bioaccumulation of pharmaceutical residues may have occurred at concentrations measuring below the method detection limits.

## 2.2 Assessment of the Ecotoxicological Effects of Novel Contaminants in the Irish Aquatic Environment on Bivalves Using a Biomarker/ Proteomic Approach

Over recent decades, biomarkers have been applied as early-warning tools to evaluate the effects of contaminants on the health of the aquatic ecosystem. Biomarkers can indicate at a cellular level the potential of stress and damage in organisms following an exposure to pollutants. However, the identification of potential contaminants is dependent on the discrimination between the natural and altered physiological variation. Along with biotic factors, abiotic factors, including salinity, temperature, food availability and air exposure during low tide for intertidal species, are known to influence the physiological response (Letendre et al., 2009) In addition, various studies have shown the influence of season, age and sex on the biomarker response. In order to successfully use biomarker techniques to evaluate pollution effects, it is essential to understand first the potential natural variation. This includes the possible variability of the biomarker response within a species population that inhabits a geographic area that features related hydrographical and climatological characteristics (Leniö and Lehtonen, 2005).

In two separate studies (Schmidt et al., 2012, 2013), baseline data of various biomarkers of stress (GST and metallothionein (MT)), damage to tissue (lipid peroxidation (LPO)) and DNA, and reproduction (vitellin-like proteins (Vn)) in marine mussels (*Mytilus* spp.) were investigated. A study from four locations along a vertical transect from high to low shore (Fig. 2.2) compared biomarker expressions in relation to vertical location with those from cultivated long-line mussels.

High shore and cultivated mussels showed significantly higher LPO and DNA damage expression than the low shore mussels (Fig. 2.3), indicating a level of oxidative stress resulting from mussel location (Schmidt et al., 2012). Significant effects on physiological end points, such as condition factor and soft tissue ration, were also found. The range of natural factors that can influence the defence mechanisms in intertidal and cultivated mussels is highly complex; however, this study provides an insight into the spatial variability of biomarkers of stress and damage using blue mussels. The results support the conclusions of

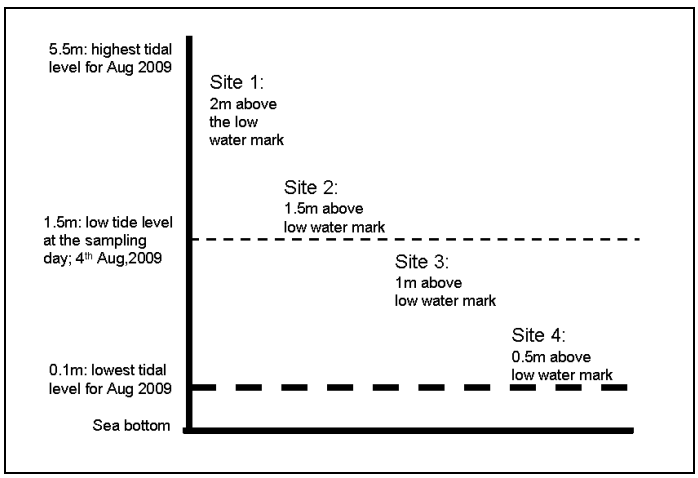



**Figure 2.2. Schematic representation of the four sampling sites with the intertidal shore location. Site 1, high shore; Site 2, middle shore; Site 3, lower middle shore; Site 4, low shore close to the sediment.**

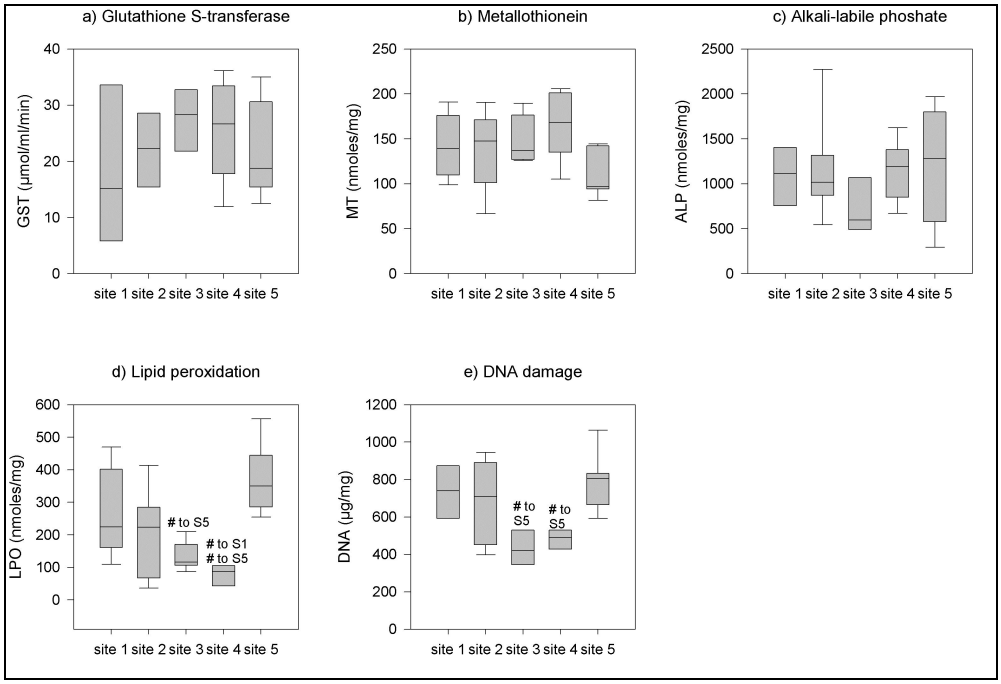



**Figure 2.3. Box plots of biomarker expression of the blue mussels (*Mytilus* spp.) at the five sites. Significance at p ≤ 0.05 (#) to the relevant site (S). (Line = median, box = 25th and 75th percentiles, whiskers = 10th and 90th percentiles).**

Letendre et al. (2009) that the location of organisms on the shore should be taken into account in sampling for ecotoxicological studies and that environmental conditions may affect antioxidant responses in mussels.

In the second study, baseline levels and the potential seasonal variation in commonly used biomarkers of stress (GST), reproduction (Vn) and damage (LPO and DNA damage) in blue mussels from a known *Mytilus* spp. hybrid zone were assessed (Schmidt et al., 2013). Levels of all biomarker expression varied between seasons (Fig. 2.4) and appeared to be linked to the reproductive cycle. Oxidative stress in winter, with low GST expression and a higher expression of LPO and DNA damage displayed could be explained by low food availability. Seasonal variations in biomarker responses are driven by the complex interaction between abiotic factors, such as temperature, salinity and food supply, and biotic factors, such as gametogenesis. These interactions complicate the interpretation of biomarker responses observed in environmental monitoring studies. Seasonal variations in biomarker responses determined in this study highlight the importance of assessing baseline levels and seasonal variations in mussel populations prior to their use in biomonitoring programmes and ecotoxicological studies. Biomarker responses measured in the current study provide vital baseline data for future studies using a native hybrid *Mytilus* population.

To investigate the potential chronic effects of exposure to gemfibrozil and diclofenac in the marine mussel *Mytilus* spp., an experiment was conducted where mussels were exposed by injection to the pharmaceuticals (Schmidt et al., 2011). Biomarker expression in the mussels was compared with standard toxicity tests using marine species (*Vibrio fischeri*, *Skeletonema costatum* and *Tisbe battaglia*i). The biomarker response of *Mytilus* spp. to gemfibrozil indicates that environmentally relevant and elevated concentrations (1 µg/l and 1,000 µg/l, respectively) stimulate oxidative stress after 24 h and 96 h exposure (Fig. 2.5). In addition, endocrine disrupting effects were shown after 24 h at 1,000 µg/l. In general a lower biomarker response was seen following exposure to diclofenac (Fig. 2.6). However, with the induction of LPO after 96 h of diclofenac exposure, the potential for tissue damage was noted. Additionally, standard

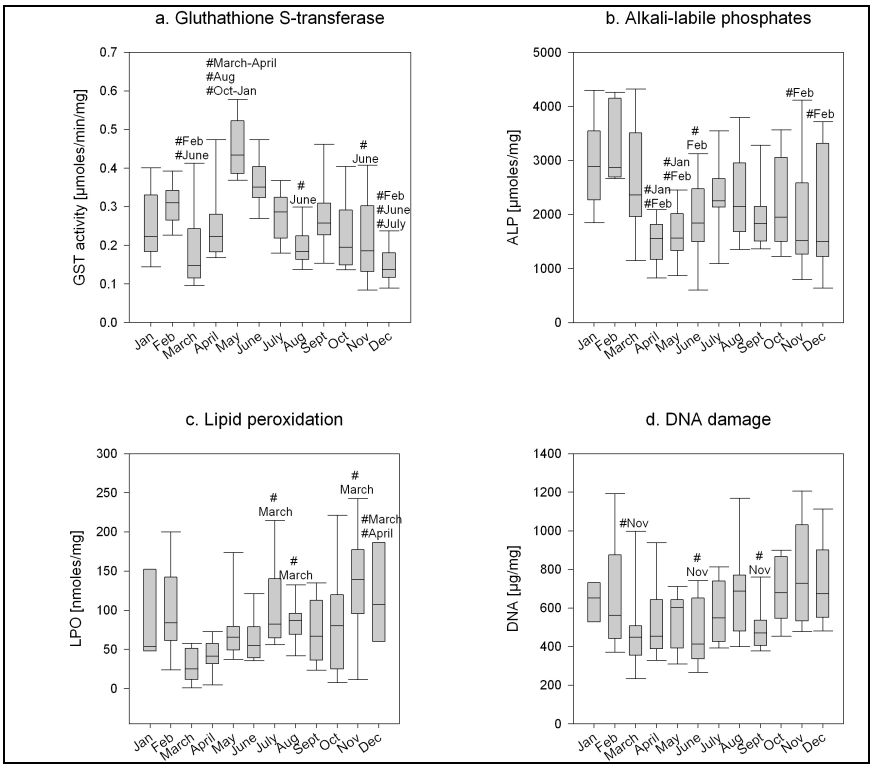



**Figure 2.4. Box plots of biomarker expression in blue mussels over the course of the year 2009. Significance at p < 0.05 with (#) different to the month indicated. (Line = median, box = 25th and 75th percentiles, whiskers = 10th and 90th percentiles).**

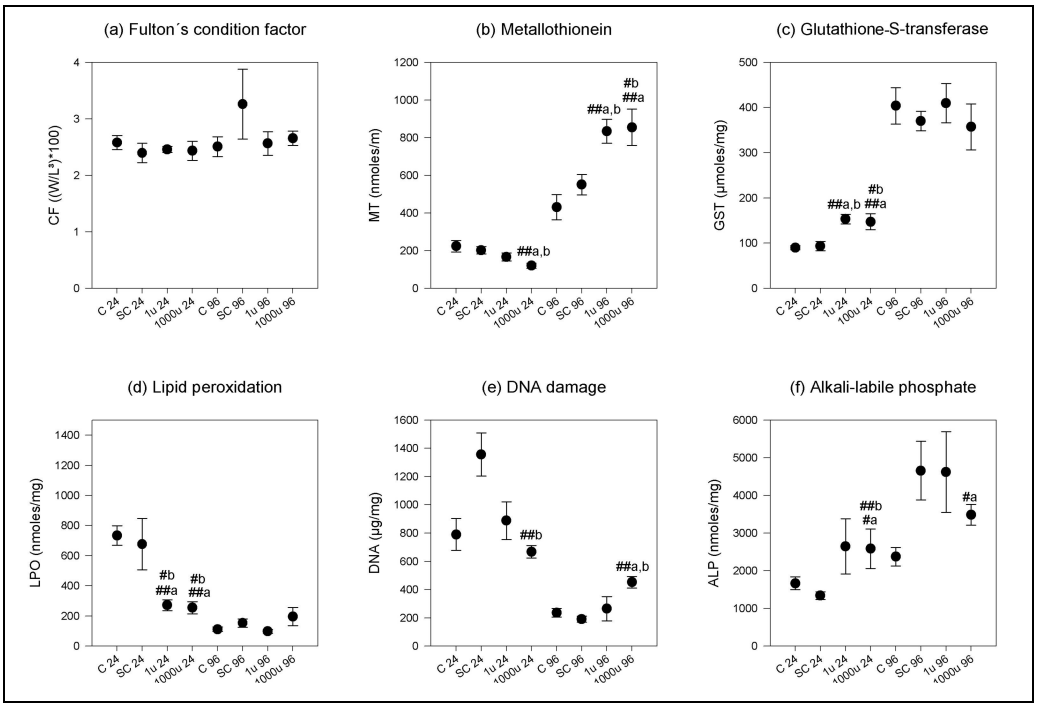



**Figure 2.5. Biomarker expression (mean ± SE) of blue mussels (*Mytilus* spp.) exposed to 1 µg/l and 1,000 µg/l gemfibrozil (•) for 24 h and 96 h. Significance set at p ≤ 0.05 (#) and p ≤ 0.01 (##) with (a) significant to control, (b) significant to solvent control.**

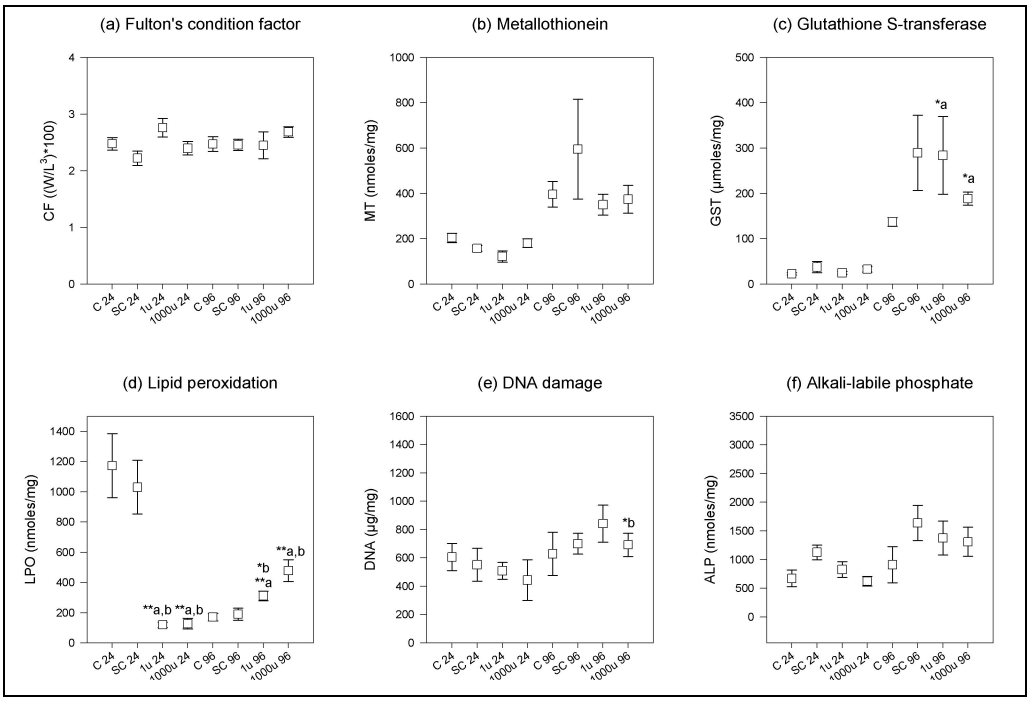



**Figure 2.6. Biomarker expression (mean ± SE) of blue mussels (*Mytilus* spp.) exposed to 1 µg/l and 1,000 µg/l diclofenac (□) for 24 and 96 h. Significance set at p ≤ 0.05 (*) and (**) at p ≤ 0.01 with (a) significant to control, (b) significant to solvent control.**

toxicity tests using the marine species showed differences in sensitivity to both drugs in the milligram/litre range. This biomarker test has demonstrated that exposures to both pharmaceuticals significantly affect detoxification and defence systems in blue mussels (Schmidt et al., 2011).

Furthermore, the acute and chronic toxicity of these compounds in freshwater species (*Dreissena polymorpha*, *Daphnia magna*, *Pseudokirchneriella subcapitata*, *Lemna minor*) was assessed (Quinn et al., 2011). For the acute end points ($IC_{50}$ and $EC_{50}$), gemfibrozil showed higher toxicity ranging from 29 to 59 mg/l (diclofenac 47–67 mg/l), while diclofenac was more toxic for the chronic *Daphnia magna* 21-day end points, ranging from 10 to 56 mg/l (gemfibrozil 32–100 mg/l). These results were compared with the expression of several biomarkers in the zebra mussel (*Dreissena polymorpha*) 24 and 96 h after exposure by injection to concentrations of 1 and 1,000 µg/l (Fig. 2.7). Exposure to gemfibrozil and diclofenac at both concentrations significantly increased the level of lipid peroxidation, a biomarker of damage. At the elevated nominal concentration of 1,000 µg/l the biomarkers of defence GST and MT were significantly elevated for gemfibrozil and diclofenac, respectively, as was DNA damage after 96 h exposure to gemfibrozil. No evidence of endocrine disruption was observed using the alkali-labile phosphate technique. Results from this suite of biomarkers indicate that these compounds can cause significant stress at environmentally relevant concentrations, acting primarily through oxidation pathways with significant destabilisation of the lysosomal membrane and that biomarker expression is a more sensitive end point than standardised toxicity tests.

However, these tests are either based on their short-term acute toxicity, which is only relevant when accidental discharge of the drug occurs or cover only particular aspects of a general stress response, making them unsuitable to identify potential effects of pharmaceuticals. Furthermore, many biomarkers are influenced by multiple confounding factors, which may limit their transfer from laboratory to field studies. Although with a proteomic approach external confounding factors (e.g. seasonality) cannot be excluded, this approach offers the advantage of a

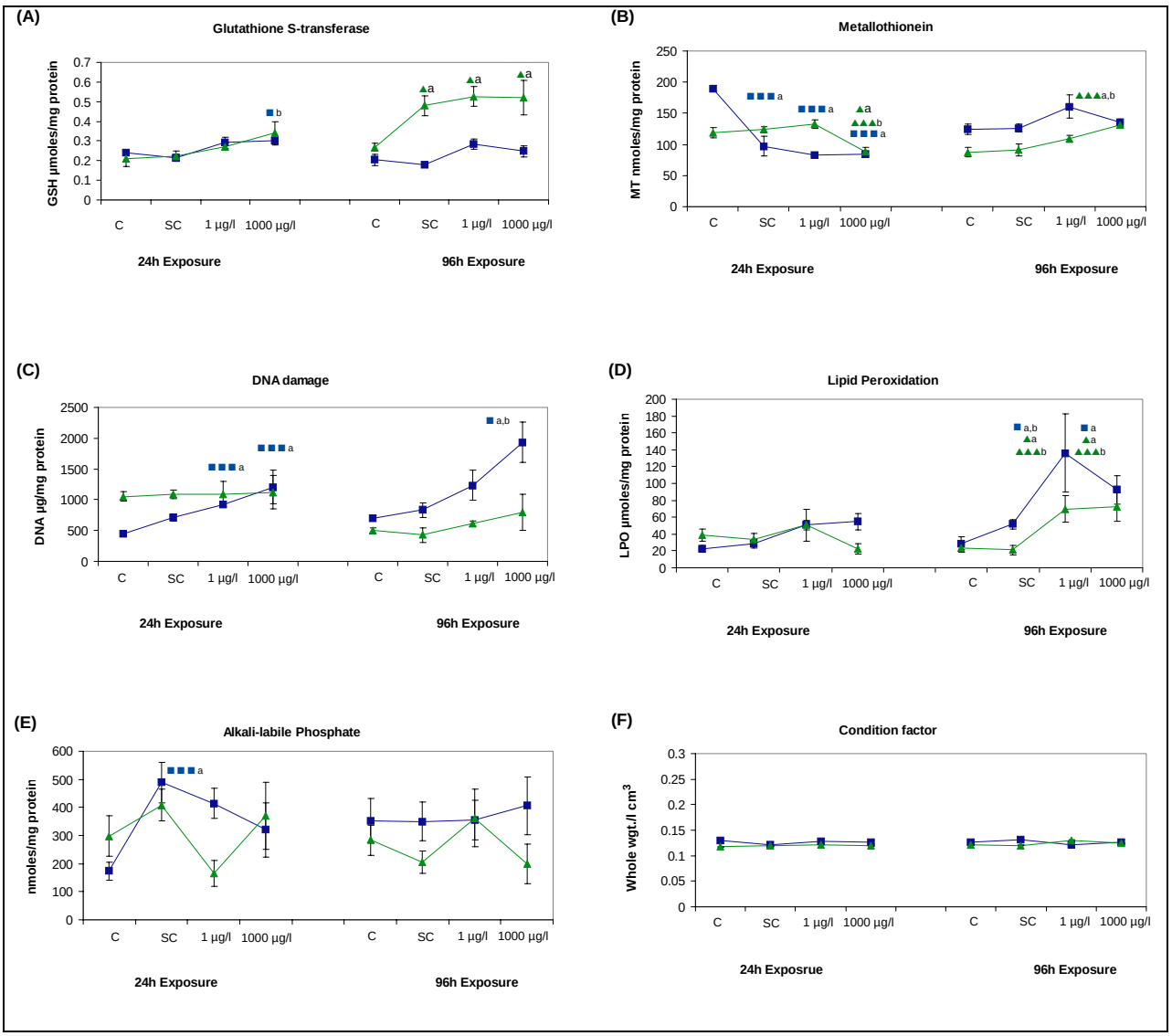



**Fig. 2.7. Biomarker expression of zebra mussels (*Dreissena polymorpha*) exposed to 1 and 1,000 µg/l gemfibrozil (■) and diclofenac (▲) for 24 and 96 h. (A) Glutathione-S-transferase; (B) Metallothionein; (C) DNA damage; (D) Lipid peroxidation; (E) Alkali-labile phosphate; (F) Condition factor. Significance set at ■, ▲ p < 0.05; ■■, ▲▲ p < 0.01; ■■■, ▲▲▲ p < 0.001, with (a) significant against control, (b) significant against solvent control.**

potentially more detailed understanding of the toxicological effects of the compounds since a large number of proteins can be analysed simultaneously. Changes in the protein expression signature (PES) of an organism or tissue can help identify specific responses to a stressor, such as a pharmaceutical (Shepard et al., 2000). This could help not only in the understanding of the mechanisms of action, but also in the recognition of novel, specific biomarkers. The study by Schmidt et al. (2014) aimed to investigate the potential chronic sublethal effects of two commonly found human pharmaceuticals, diclofenac and gemfibrozil, on *Mytilus* spp. exposed for 14 days to environmentally relevant and elevated concentrations of the compounds (1 µg/l and 1,000 µg/l, respectively). In addition, mussels were maintained for an extra week to investigate the potential for recovery from exposure. Chronic sublethal effects were examined by investigating differential PESs of the digestive gland of *Mytilus* spp. as well as by the determination of biomarkers of effect after 7, 14 and 21 days. In summary, using two-dimensional gel electrophoresis,

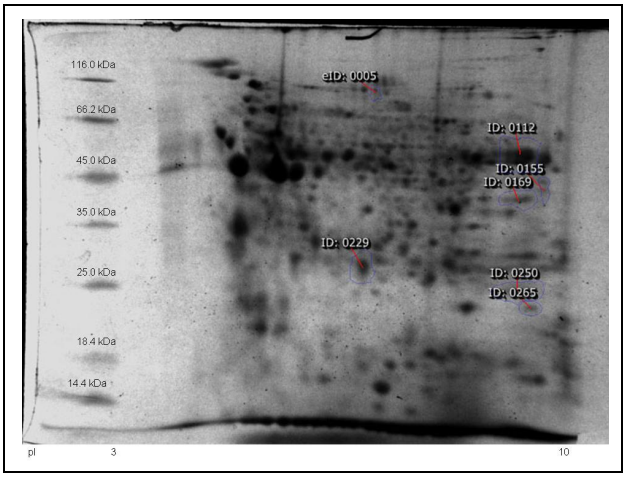



**Figure 2.8. Annotated two-dimensional gel electrophoresis image with the seven spots identified by LC-MS/MS (marked with their ID number). The annotated spots were selected by combined PCA and ANOVA ($p \leq 0.05$ and with a fold change > 2).**

12 isolated protein spots were significantly increased or decreased by gemfibrozil and/or diclofenac, seven of which were successfully identified by LC-MS/MS analysis (Fig. 2.8). These proteins were involved in energy metabolism, oxidative stress response, protein folding and immune responses. Changes in the PES over time suggested that mussels were still experiencing oxidative stress for up to 7 days post-exposure. In addition, a suite of biomarkers comprising GST, lipid peroxidation and DNA damage was studied. An oxidative stress response was confirmed by biomarker responses. To the authors' knowledge, this is the first investigation using proteomics to assess the potential effects of these pharmaceuticals on a non-target species in an environmentally relevant model. The successful application of this proteomic approach supports its potential use in pollution biomonitoring and highlights its ability to aid in the discovery of new biomarkers.

## 2.3 Development and Use of in Vitro Toxicity Tests to Assess the Acute Effects of Pharmaceuticals on Aquatic Bivalves

Despite the successful transfer of mammalian in vitro techniques for use with fish and other vertebrates, little progress has been made in the area of invertebrate tissue culture. As part of this project, Quinn et al. (2009) describe the development of an in vitro technique for the culture of both cells in suspension and tissue explants from the gill, digestive gland and mantle of the zebra mussel (*Dreissena polymorpha*) (Fig. 2.9) and their successful maintenance in culture for up to 14 days. Cell suspensions from the gills and digestive gland were the most successful technique developed, with a viability of >80% maintained for up to 8 days in culture, making it suitable for use in short-term toxicity tests. Tissue explants from the mantle were also maintained in culture for up to 14 days. This paper (Quinn et al., 2009) describes the challenges involved in the development of a novel in vitro culture technique for aquatic invertebrates.

The study by Parolini et al. (2011) investigated the in vitro cytotoxicity of four common drugs, namely atenolol, carbamazepine, diclofenac and gemfibrozil, on three different cell typologies from *Dreissena polymorpha*: haemocytes, gill and digestive gland cells. Results obtained by the Trypan Blue Exclusion Test revealed that exposure to increasing concentrations (0.001, 0.01, 0.1, 1 and 10 mg/l) of carbamazepine, diclofenac and gemfibrozil significantly decreased the viability of each cell type, while the 3(4,5-dimethyl-2thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) reduction assay highlighted only a slight reduction in mitochondrial activity of gill and digestive gland cells. Overall, diclofenac was the most cytotoxic drug for zebra mussel cells, followed by gemfibrozil, carbamazepine, while atenolol had no noteworthy toxic potential (Table 2.2). These results

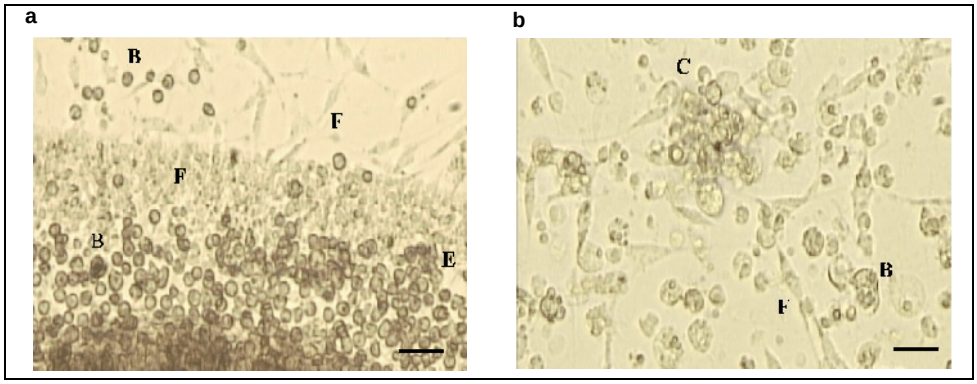



**Figure 2.9. (a) Explant of mantle from the zebra mussel after 8 days in culture showing migration of fibroblast-like (F) and ball-shaped (B) cells from the explant (E). Magnification × 200. Scale bar = 100 µm. (b) Dissociated gill cells from the zebra mussel after 8 days in culture, cells have aggregated into cell clumps (C) with fibroblast-like cells (F) and ball-shaped cells (B) attached to the substrate. Magnification × 400. Scale bar = 50 µm.**

**Table 2.2. 96 h Effect concentration 50% (96 h $EC_{50}$) and corresponding confidence limit (95% CL) values for gill, digestive gland cells and haemocytes for in vitro exposure to carbamazepine, diclofenac and gemfibrozil calculated using the Trimmed Spearman–Karber method. For atenolol $EC_{50}$ values were not calculable (n.c.).**

| | $LC_{50}$ (95% confidence limits) | | | |
|---|---|---|---|---|
| | **Carbamazepine (mg/l)** | **Diclofenac (mg/l)** | **Gemfibrozil (mg/l)** | **Atenolol** |
| **Gill cells** | 5.09 (1.03–25.08) | 8.55 (2.0–36.53) | 3.37 (1.05–10.83) | n.c. |
| **Digestive gland cells** | 6.78 (1.10–41.66) | 6.22 (1.59–24.4) | 6.32 (2.7–14.76) | n.c. |
| **Haemocytes** | 5.29 (1.26–22.30) | 0.82 (0.13–5.11) | 6.57 (2.01–21.49) | n.c. |

lay the groundwork for further in vitro evaluations, which will allow a better definition of the potential toxicity of these drugs.

Although the results of these studies (Quinn et al., 2009; Parolini et al., 2011) are somewhat preliminary, a considerable amount of further work is still needed in the area of invertebrate tissue culture. In vitro biomarker expression (GST induction) was also investigated and observed in gill and digestive gland explants of the zebra mussel exposed to the pharmaceutical drug, diclofenac, and the common polycyclic aromatic hydrocarbon, benzo(a)pyrene (Fig. 2.10). It is hoped that the adaptation of this technique (Fig. 2.11) to the marine mussel *Mytilus edulis* will be relatively straightforward, using the same basic techniques and adjusting the media and reagents used to better resemble the osmolarity and salt content of haemolymph of this marine species. The ultimate aim of this research is to gain the ability to investigate chronic end points in vitro that would represent and hopefully replace the need for whole animal in vivo exposures, thus saving on the time, resources and number of animals needed to undertake toxicity tests to meet the requirements of monitoring legislation such as REACH[2].

2. REACH is the Regulation for Registration, Evaluation, Authorisation and Restriction of Chemicals.

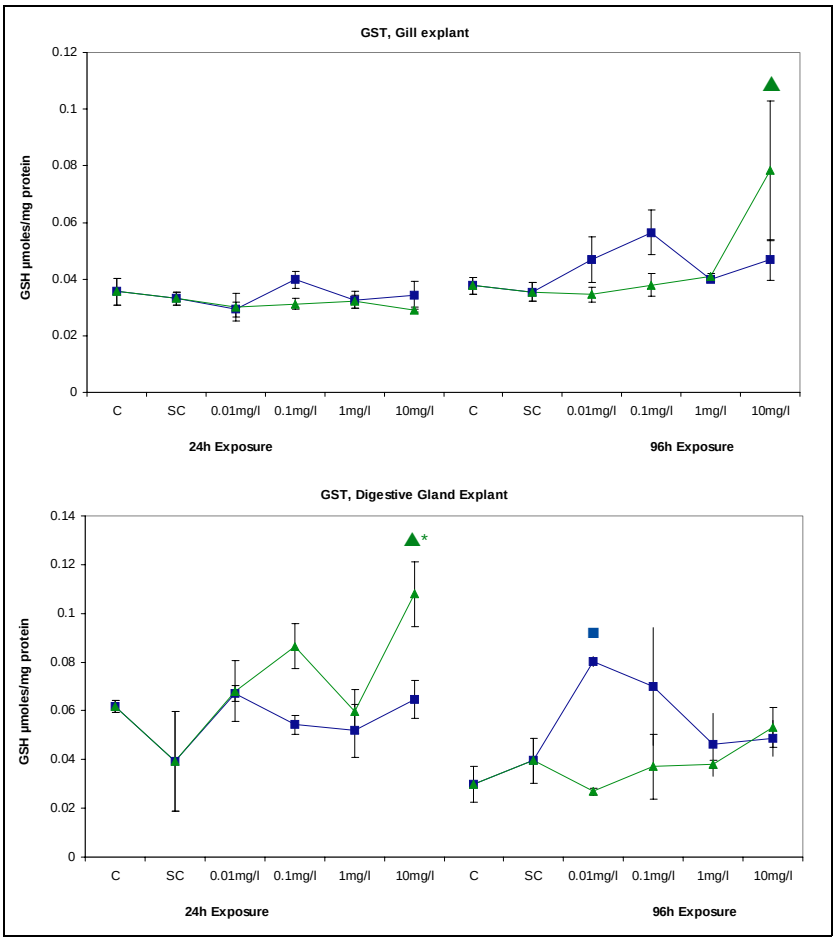



**Figure 2.10. Glutathione-S-transferase (GST) expression in gill and digestive gland explants from *Dreissena polymorpha* exposed *in vitro* to diclofenac (■) and benzo(a)pyrene (▲) for 24 and 96 h. Data expressed as mean (n = 3) ± SE. Significance was calculated against the control (C) and solvent control (SC) using one way ANOVA. Significance at ■ p < 0.05,diclofenac; ▲ p < 0.05, benzo(a)pyrene. *Significant to SC only.**

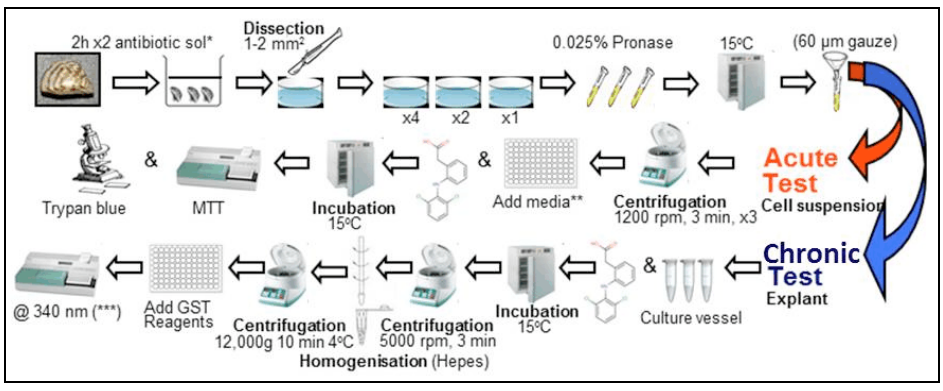



**Figure 2.11. A visualisation of the method developed for the culture of cell suspensions and tissue explants from the zebra mussel (*Dreissena polymorpha*) and their use in acute and chronic toxicity testing (respectively). *Antibiotic solution. **Culture media as described by Quinn et al. (2009). ***GST measurement based on method of Boryslawskyi et al. (1988). MTT, 3(4,5-dimethyl-2thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; GST, glutathione-S-transferase.**

## 2.4 Environmental Diagnostics: the Use of Human Diagnostic Techniques for Environmental Monitoring

One of the goals of this EPA DERP-funded project was the development of diagnostic tools that could be used for environmental monitoring by government agencies such as the EPA or the Marine Institute. In a productive collaboration between the Irish Centre for Environmental Toxicology (ICET) and Cruinn Diagnostics Ltd (Dublin), a translational approach was adapted to investigate the application of human diagnostic techniques to assess the health of animals exposed to various environmental stressors, potentially providing a valuable tool for environmental monitoring. Since many of the end points (steroid and clinical chemistry end points) that they measure have been evolutionarily conserved throughout the phyla, the adaptation of these techniques for measuring the health of non-human samples should be relatively straightforward. Once the presence and measurement of these end points has been established and validated in non-human samples, these techniques could allow a fast (high throughput), efficient, cost-effective and validated method that is fully (or partially) automated and can be operated by a non-specialist. This approach could allow for the development of a comprehensive quality assurance programme to ensure compatibility of data, something that is currently lacking in many biomarker studies. Much of this work was undertaken in the final year of the DERP project. Although the work has been undertaken, the results are not yet fully analysed and have not yet been published. For this reason, the specific results from the environmental diagnostic work unfortunately cannot be presented in this report. However, a general trend in the preliminary results is briefly summarised below.

The Siemens Immulite 2000 Immunoassay Analyser was used to measure background levels of steroids (oestrogen, follicle stimulating hormone, luteinising hormone, testosterone, adrenocorticotropic hormone and progesterone) in marine (*Mytilus* spp.) and freshwater (*Dreissena polymorpha*) bivalves and to evaluate the impact of exposure to potential endocrine disrupting chemicals (EDCs), 17α-ethynylestradiol and municipal effluent. The aims of this study were to:

- Investigate if human diagnostic methods can be used for assessment in non-target species;
- Provide important background information on the levels of the steroids over a 12-month period; and
- Investigate the impact of exposure to potential EDCs, 17α-ethynylestradiol and municipal effluent on the levels of steroids in these animals.

Preliminary data analysis indicates that steroid levels were found to be more stable and measureable throughout the year in the digestive gland and to fluctuate greatly in the gonad, which is heavily influenced by the point in the reproductive cycle (Fig. 2.12). Following a 7-day exposure of *Mytilus edulis* to 17α-ethynylestradiol, significant changes in steroid expression were observed and found to be related to vitellogenin induction as measured by the indirect alkali-labile phosphate biomarker. As none of these results have yet been published, they unfortunately cannot be discussed in more detail.

The Piccolo xpress$^{TM}$ clinical chemistry analyser (Fig. 2.13) was used to analyse clinical chemistry end points (alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, gamma glutamyltransferase, total bilirubin, amylase, creatinine, glucose and uric acid) in numerous bird, fish and invertebrate species.

All species tested positive for these end points. It is hoped that these techniques will allow the investigation of the health of animals and the environmental impact of pollutants upon animals from various trophic levels, from invertebrates to vertebrates, including fish, birds and marine mammals. This could offer a comprehensive picture of the health of animals or the environmental impact of pollutants on an entire ecosystem using the same validated, standardised method to investigate ecologically relevant end points. Preliminary data indicate that in an exposure of bivalves (*Mytilus* spp.) and fish (*Onchrhynchus mykiss*) to diclofenac, significant changes in alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase and amylase (used to evaluate hepatocellular and pancreatic injury) occurred at environmentally relevant and elevated concentrations of 1 and 1,000 µg/l, respectively (Fig. 2.14).

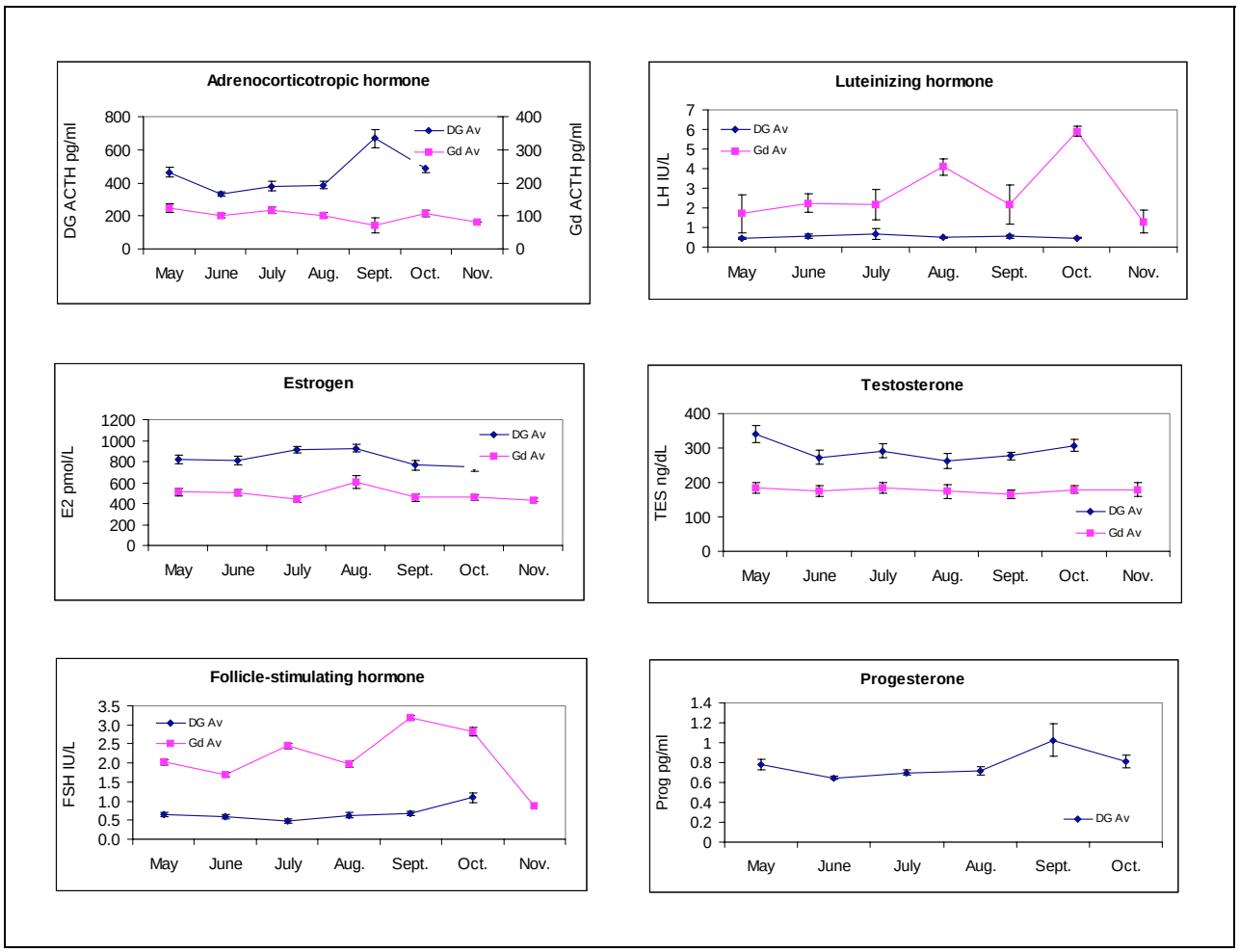



**Figure 2.12. Preliminary data set of steroid levels (May–October/November) measured in the marine blue mussel (*Mytilus* spp) (n = 8) using the Siemens Immulite 2000 Immunoassay Analyser. DG, digestive gland; Gd, gonad.**

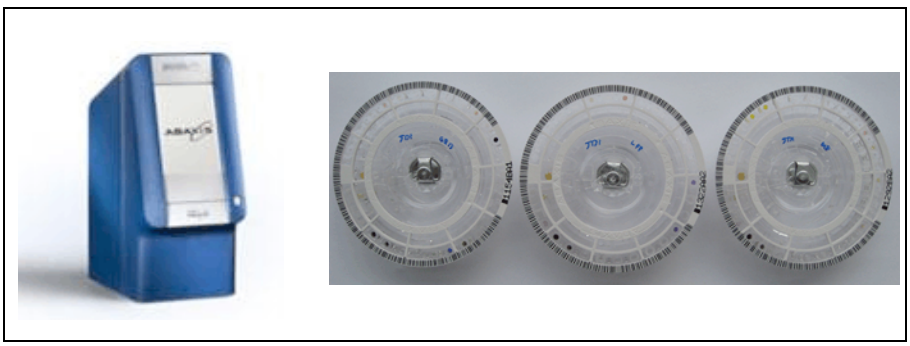
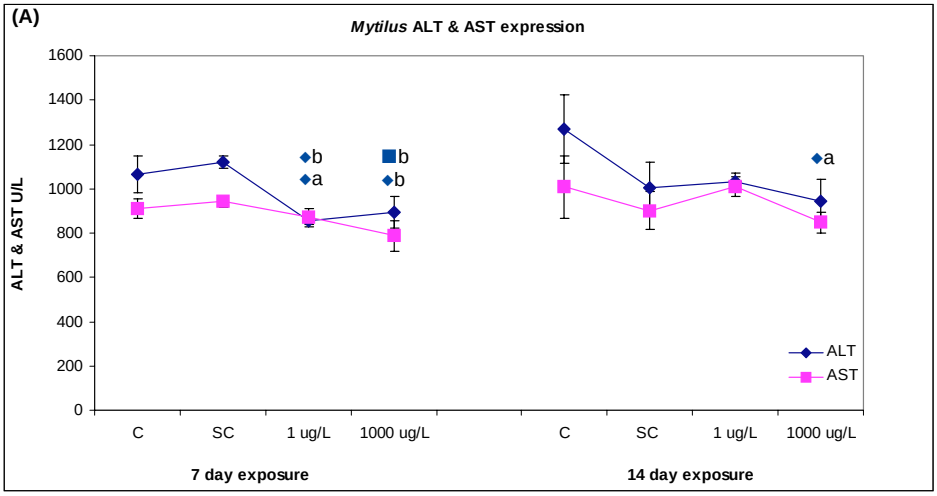
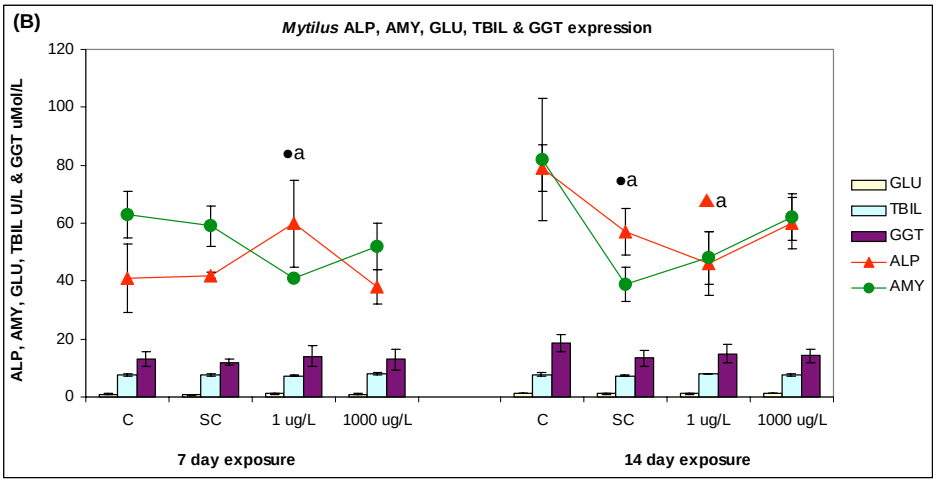


**Fig. 2.13. Image and description of the Piccolo xpress$^{TM}$ clinical chemistry analyser and of the disposable GC13 rotors used for this work.**

It is hoped that the development and validation of a protocol in this study will help increase the use of mussel haemolymph to assess the health status of these animals, using human-based diagnostic tools, as a sublethal early-warning indication of pollution.



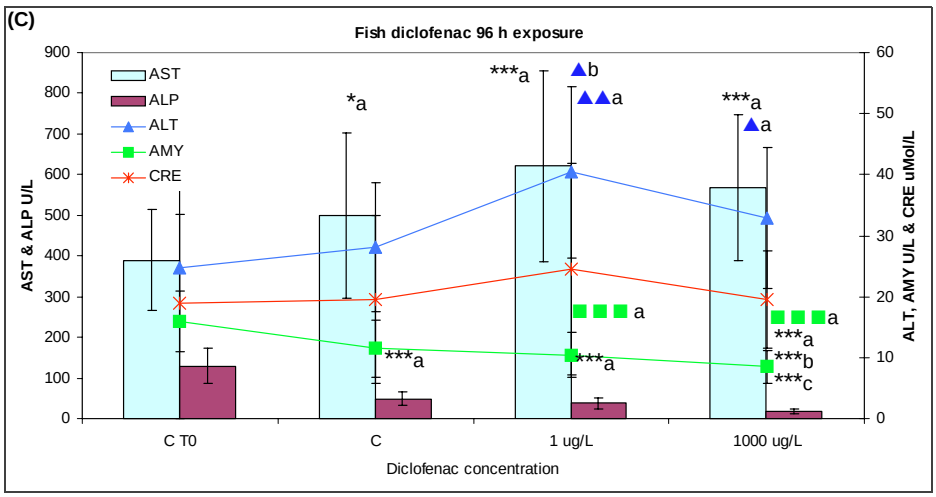




**Fig. 2.14. (A and B) Clinical chemistry results of digestive gland homogenate S15 from mussels exposed to diclofenac for 7 and 14 days. a & b = significant against control (C) and solvent control (SC), respectively. (C) Clinical chemistry results for blood plasma from rainbow trout exposed to diclofenac for 96 h, with (a), (b) and (c) = significant against CT0, C and 1 µg/l, respectively. CT0, control time 0.**

# 3 Summary of Overall Project

## 3.1 Project Output

The output from this project includes new information, the development of new methods and increased awareness. The project has built a capacity for and developed new partnerships focused on research into pharmaceuticals as environmental contaminants.

## 3.2 New Information

A summary of the research outputs from this project is presented in Appendix 3. These outputs include the following:

- The first report on spatial occurrence and relative distribution of pharmaceutical residues in the Irish marine environment and their potential to bioaccumulate in marine fauna.
- The ability of pharmaceutical concentrations in marine mussels to increase after cooking and their potential for human exposure.
- Establishment and publication of vital baseline data on biomarker response in introgressed *Mytilus* spp. in order to better interpret responses observed following exposure to contaminants.
- The acute and chronic toxic impact of pharmaceuticals on both fish and numerous invertebrate and algal species, particularly focusing on biomarker expression.
- The development of invertebrate in vitro methods and their potential for the toxicity testing of pharmaceuticals.
- The adaptation of human diagnostic techniques for environmental monitoring.

## 3.3 New Methods

- A validated method for the analysis of five pharmaceutical compounds in municipal effluent, marine receiving waters and biological tissue using PLE and SPE with LC-MS/MS.
- Application of ‘omics’ technologies in an environmental proteomic approach to detect a unique PES of blue mussels in response to exposure to the potential adverse effects of the novel contaminants diclofenac and gemfibrozil.
- Development of invertebrate in vitro cell suspension and tissue explant culture techniques for use in cytotoxicity and sublethal end points, respectively.
- Protocols developed for the use of tissue and haemolymph from the marine blue mussel (*Mytilus* spp.) for environmental diagnostic monitoring using human diagnostic techniques.

## 3.4 Capacity Building

The project has provided research and training to eight undergraduate and two postgraduate students, building the capacity for environmental research and environmental protection in Ireland.

## 3.5 Partnerships for Environmental Research

The project has also directly led to the establishment of the ICET in the GMIT, through a Memorandum of Understanding between the National University of Ireland Galway, Athlone Institute of Technology and the GMIT. This helped foster a positive relationship and enabled increased collaboration between these institutes.

# 4 Conclusions from Research and Future Research Needs

- It is clear from the results generated during this project that pharmaceutical residues are present in quite substantial quantities in the Irish aquatic environment when compared with marine receiving waters in other western countries. It has been shown that a number of commonly used pharmaceuticals persist beyond the wastewater treatment process and enter receiving surface waters. This research has highlighted the ability of marine mussels to bioaccumulate low-level pharmaceutical concentrations present in their surrounding aquatic environment.

- Due to the ability of these compounds to readily metabolise, there is a need to combine both chemical and biological analyses, particularly for the assessment of pharmaceutical uptake in naturally exposed aquatic organisms, as seen in the case where low uptake does not necessarily indicate low exposure or risk.

- Chemical analysis in this area still proves to be very challenging, primarily due to the complexity of the environmental sample matrices involved, in particular the co-extraction of the sample matrix with selected analytes, adversely affecting method sensitivity and detection, although expensive deuterated standards and new separation technologies such as ultra high pressure liquid chromatography (UHPLC) can be used to address this problem.

- Based on the results from this and other chronic toxicity studies, the pharmaceutical monitoring data produced for carbamazepine and diclofenac within this project highlight a potential risk for chronic effects in aquatic species residing in the selected Irish coastal zones.

- It was observed that the European maximum residue limit (MRL) for trimethoprim in all food-producing species (50 ng/g) was not exceeded in marine mussels collected from one of the most highly contaminated sites in Ireland, deeming them safe for human consumption.

- Four of the five selected pharmaceuticals showed an increase in concentration in the marine mussels after cooking, with exceptionally high increases measured for acidic pharmaceuticals in particular. Although diclofenac and several other acidic pharmaceuticals are not monitored in seafood, they are controlled in other foodstuffs in the EU. The research referred to in Section 2.3 highlighted the possibility for pharmaceutical residues in approved foodstuffs to exceed the assigned MRL values following thermal treatment of the food, potentially posing a risk to human health via dietary ingestion.

- Results indicate that these acute toxicity tests are not sufficiently sensitive to predict adverse effects at low environmentally relevant concentrations for pharmaceuticals in the environment.

- The information generated within this research provides crucial background data for use in future ecotoxicological studies, minimising the influence of confounding factors, maximising the interpretation of biomarker responses and facilitating the discrimination between natural and anthropogenic stress. Furthermore, these data are an important step for Ireland's future integrated environmental monitoring using biomarker techniques in combination with chemical analysis.

- This work indicates that human diagnostic techniques could potentially offer cost-effective, quick and validated methods to assess chronic impacts of environmental contaminants.

# 5 Recommendations for Implementation and Uptake of Research Findings

The following areas have been identified as requiring further research:

- Besides encouraging doctors against the unnecessary prescribing of pharmaceuticals and providing a greater awareness of correct ways of disposing of unused or out-of-date pharmaceuticals, there is no alternative to reduce the presence of pharmaceuticals in sewerage systems. Removal technologies for pharmaceuticals should be employed in WWTPs to prevent the release of potentially harmful pharmaceuticals into the environment; however, emission limits first need to be set before the most cost-effective treatments can be implemented.

- As pharmaceuticals do not occur isolated in the natural environment, further study is required to assess the ecological relevance and ecotoxicological potential of prevalent pharmaceutical mixtures in the aquatic environment. This should include drug metabolites in analytical methods, especially for the likes of prodrugs, which release the active compound after undergoing metabolism.

- The possibility for antibiotics, such as trimethoprim, to bioaccumulate in aquatic species and potentially through the food chain was highlighted. A more in-depth study of the fate of persistent antibiotics in the aquatic environment should be carried out and their abilities to bioaccumulate in other aquatic species further assessed.

- Chronic toxicity studies need to be carried out on a wider range of aquatic species and should include exposure to pharmaceutical concentrations lower than 1 µg/l in order to produce predicted no-effect concentration (PNEC) data and allow for the accurate environmental risk assessment of pharmaceuticals.

- Following the results of this project, it may be necessary to further investigate animal food products, containing pharmaceutical residues at concentrations close to the defined MRL values after thermal treatment (or cooking).

- The risks for the aquatic environment, associated with the measured water concentrations of the targeted pharmaceuticals within this study, cannot be fully assessed as there are currently no regulatory guidelines established for widely used and widespread occurring pharmaceuticals. This knowledge gap needs to be addressed.

- Further research is required to investigate the potential toxic effects of these compounds in additional marine species in order to elucidate differences in species sensitivity. It is recommended that future tests use a number of species from several different taxa in order to better understand the potentially negative effects of newly emerging contaminants.

- The majority of published pharmaceutical toxicity data is for freshwater organisms. The inclusion of more marine species in standardised toxicity tests is recommended. This is particularly important in order to meet requirements set out by the WFD for coastal and transitional waters, and by the MSFD for marine waters. The integration of the biomarker effects with standardised toxicity tests would increase the accuracy of toxicity assessment of pharmaceuticals.

- The comparison between background concentrations and altered biomarker expressions shows that an unambiguous evaluation of the biomarker results remains

challenging, and further studies to build on this research are recommended.

- The application of human diagnostic techniques for environmental monitoring in an area termed 'environmental diagnostics' holds great potential for environmental monitoring. The establishment of validated protocols is recommended to enable the proper development of these techniques.

# References


Barron, L., Tobin, J. and Paull, B., 2008. Multi-residue determination of pharmaceuticals in sludge and sludge enriched soils using pressurized liquid extraction, solid phase extraction and liquid chromatography with tandem mass spectrometry. *Journal of Environmental Monitoring* **10(3):** 353–361.

Barron, L., Purcell, M., Havel, J., Thomas, K., Tobin, J.M. and Paull, B., 2009. *Occurrence and Fate of Pharmaceuticals and Personal Care Products within Sewage Sludge and Sludge-Enriched Soils*. Environmental Protection Agency, Johnstown Castle Estate, Wexford, Ireland.

Boryslawskyi, M., Garrod, A.C., Pearson, J.T. and Woodhead, D., 1988. Elevation of glutathione-S-transferase activity as a stress response to organochlorine compounds in the freshwater mussel, *Sphaerium Corneum*. *Marine Environmental Research* **24:** 101–104.

Brausch, J.M., Connors, K.A., Brooks, B.W. and Rand, G.M., 2012. Human pharmaceuticals in the aquatic environment: a review of recent toxicological studies and considerations for toxicity testing. *Reviews of Environmental Contamination and Toxicology* **218:** 1–99.

Daughton, C.G., 2007. Pharmaceuticals in the environment: sources and their management. *In:* Barceló, D. (Ed.) *Wilson & Wilson's Comprehensive Analytical Chemistry: Analysis, Fate And Removal Of Pharmaceuticals In The Water Cycle.* Elsevier, Amsterdam, The Netherlands.

EEA, 2010. *Pharmaceuticals in the Environment. Results of an EEA Workshop*. European Environment Agency, Copenhagen, Denmark.

EU, 2013. European Union Water Framework Directive 2013/39/Eu. *Official Journal of the European Communities*, OJL226.

IPHA, 2012. *Pharmaceutical Healthcare Facts and Figures*. Irish Pharmaceutical Healthcare Association, Dublin, Ireland.

Kimbrough, K.L., Johnson, W.E., Lauenstein, G.G., Christensen, J.D. and Apeti, D.A. (Eds), 2008. *An Assessment of Two Decades of Contaminant Monitoring in the Nation's Coastal Zone*. NOAA Technical Memorandum, Silver Spring, MD, USA. pp. 1–105.

Lacey, C., McMahon, G., Bones, J., Barron, L., Morrissey, A. and Tobin, J.M., 2008. An LC-MS method for the determination of pharmaceutical compounds in wastewater treatment plant influent and effluent samples. *Talanta* **75:** 1089–1097.

Leniö, S. and Lehtonen, K.K., 2005. Seasonal variability in biomarkers in the bivalves *Mytilus edulis* and *Macoma balthica* from the northern Baltic Sea. *Comparative Biochemistry and Physiology, Part C* **140:** 408–421.

Letendre, J., Chouquet, B., Manduzio, H., Marin, M., Bultelle, F., Leboulenger, F. and Duran, F., 2009. Tidal height influences the levels of enzymatic antioxidant defences in *Mytilus edulis*. *Marine Environmental Research* **67:** 69–74.

McEneff, G., Barron, L., Kelleher, B., Paull, B. and Quinn, B., 2013. The determination of pharmaceutical residues in cooked and uncooked marine bivalves using pressurised liquid extraction, solid phase extraction and liquid chromatography–tandem mass spectrometry. *Analytical and Bioanalytical Chemistry* **405:** 9509–9521.

McEneff, G., Barron, L., Kelleher, B., Paull, B. and Quinn, B., 2014. A year-long study of the spatial occurrence and relative distribution of pharmaceutical residues in sewage effluent, receiving marine waters and marine bivalves. *Science of the Total Environment* **476–477:** 317–326.

Parolini, M., Quinn, B., Binelli, A. and Provini, A., 2011. Cytotoxicity assessment of four pharmaceutical compounds on the zebra mussel (*Dreissena polymorpha*) haemocytes, gill and digestive gland primary cell cultures. *Chemosphere* **84:** 91–100.

Quinn, B., Costello, M., Dorange, G., Wilson, J. and Mothersill, C., 2009. Development of an in vitro culture method for cells and tissues from the zebra mussel (*Dreissena polymorpha*). *Cytotechnology* **59:** 121–134.

Quinn, B., Schmidt, W., O'Rourke, K. and Hernan, R., 2011. Effects of the pharmaceuticals gemfibrozil and diclofenac on biomarker expression in the zebra mussel (*Dreissena polymorpha*) and their comparison with standardised toxicity tests. *Chemosphere* **84:** 657–663.

Santos, L.H.M.L.M., Araújo, A.N., Fachini, A., Pena, A., Delerue-Matos, C. and Montenegro, M.C.B.S.M., 2010. Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. *Journal of Hazardous Materials* **175:** 45–95.

Schmidt, W., O'Rourke, K., Hernan, R. and Quinn, B., 2011. Effects of the pharmaceuticals gemfibrozil and diclofenac on the marine mussel (*Mytilus* spp.) and

their comparison with standardized toxicity tests. *Marine Pollution Bulletin* **62:** 1389–1395.

Schmidt, W., O'Shea, T. and Quinn, B., 2012. The effect of shore location on biomarker expression in wild *Mytilus* spp. and its comparison with long line cultivated mussels. *Marine Environmental Research* **80:** 70–76.

Schmidt, W., Power, E. and Quinn, B., 2013. Seasonal variations of biomarker responses in the marine blue mussel (*Mytilus* spp.). *Marine Pollution Bulletin* **74:** 50–55.

Schmidt, W., Rainville, L.-C., McEneff, G., Sheehan, D. and Quinn, B., 2014. A proteomic evaluation of the effects of the pharmaceuticals diclofenac and gemfibrozil on marine mussels (*Mytilus* spp.): evidence for chronic sublethal effects on stress-response proteins. *Drug Testing and Analysis* **6:** 210–219.

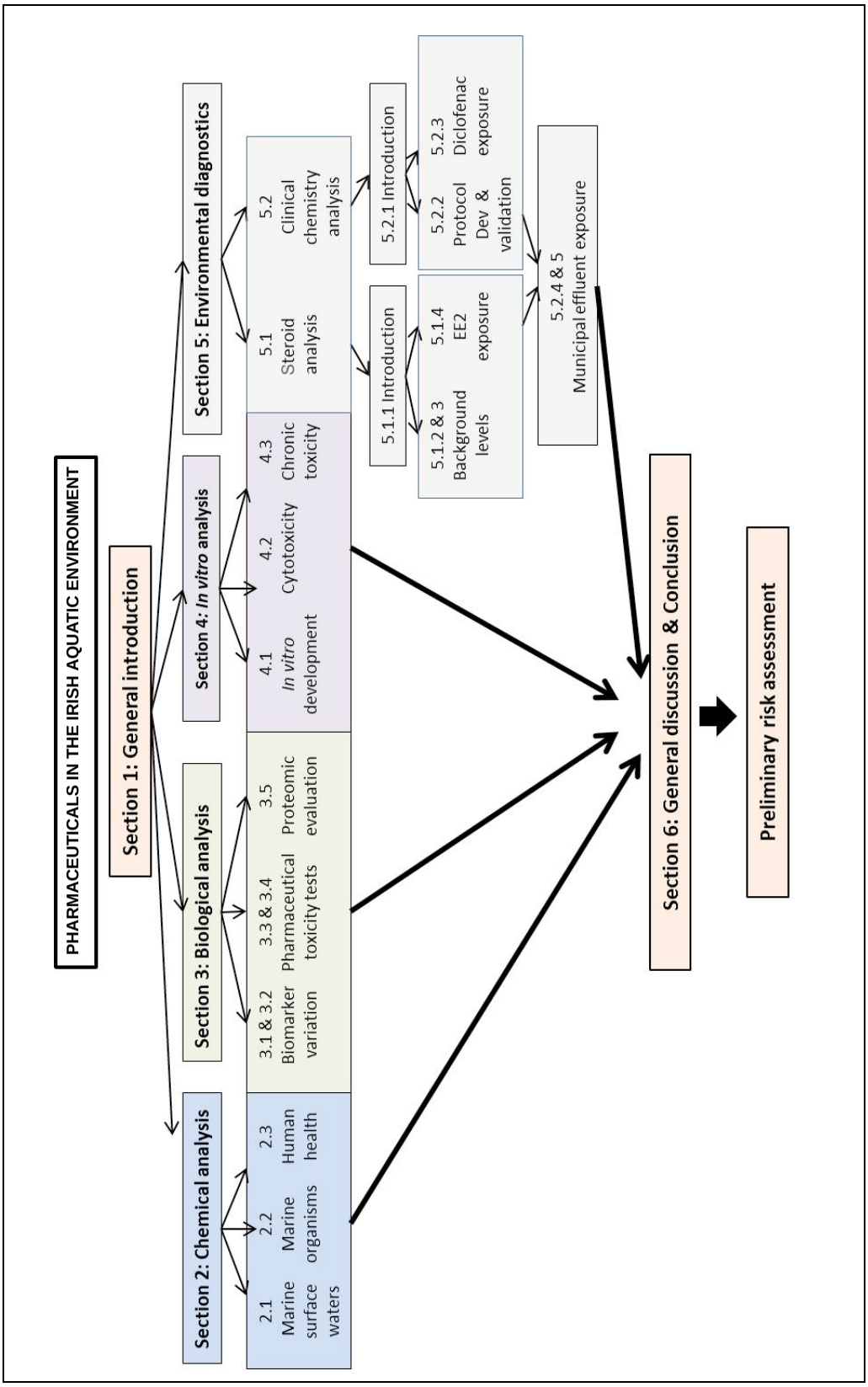
Shepard, J.L., Olsson, B., Tedengren, M. and Bradley, B.P., 2000. Protein expression signatures identified in *Mytilus edulis* exposed to PCBS, copper and salinity stress. *Marine Environmental Research* **50:** 337–340.

# Acronyms

| | |
|---|---|
| **ANOVA** | Analysis of variance |
| **ASW** | Artificial sea water |
| **CEC** | Contaminant of emerging concern |
| **CL** | Confidence limit |
| **DCU** | Dublin City University |
| **DERP** | Developing Environmental Research Potential |
| **DNA** | Deoxyribonucleic acid |
| **DW** | Dry weight |
| **EC** | European Commission |
| **$EC_{50}$** | Half maximal effective concentration |
| **EDC** | Endocrine disrupting chemical |
| **EPA** | Environmental Protection Agency |
| **EU** | European Union |
| **GES** | Good Environmental Status |
| **GMIT** | Galway–Mayo Institute of Technology |
| **GST** | Glutathione-S-transferase |
| **HPRA** | Health Products Regulatory Authority |
| **$IC_{50}$** | Half maximal inhibitory concentration |
| **ICET** | Irish Centre for Environmental Toxicology |
| **IMB** | Irish Medicines Board |
| **IPHA** | Irish Pharmaceutical Healthcare Association |
| **ISSC** | Irish Separation Science Cluster |
| **LC-MS/MS** | Liquid chromatography–tandem mass spectrometry |
| **LOL** | Limit of quantification |
| **LPO** | Lipid peroxidation |
| **MRL** | Maximum residue limit |
| **MSFD** | Marine Strategy Framework Directive |
| **MT** | Metallothionein |
| **MTT** | 3(4,5-Dimethyl-2thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide |
| **PCA** | Principal component analysis |
| **PES** | Protein expression signature |
| **PLE** | Pressurised liquid extraction |

| | |
|---|---|
| **PNEC** | Predicted no-effect concentration |
| **REACH** | REACH is the Regulation for Registration, Evaluation, Authorisation and Restriction of Chemicals |
| **SE** | Standard error |
| **SPE** | Solid phase extraction |
| **UHPLC** | Ultra high pressure liquid chromatography |
| **Vn** | Vitellin-like proteins |
| **WFD** | Water Framework Directive |
| **WWTP** | Wastewater treatment plant |

# Appendix 1 Outline of full EPA 2007-DRP-3-S5 Report



# Appendix 2 List of Project Contributors

- Prof. David Sheehan, Proteomics Research Group, Department of Biochemistry & Environmental Research Institute, University College Cork, Cork, Ireland.
- Dr Louis-Charles Rainville, Proteomics Research Group, Department of Biochemistry & Environmental Research Institute, University College Cork, Cork, Ireland.
- Dr Damian Griffin, Department of Clinical Biochemistry, University College Hospital Galway, Ireland.
- Dr Ana Varela Coelho, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras, Portugal.
- Prof. Martin Cormican, Ryan Institute, National University of Ireland Galway, Galway, Ireland.
- Dr Marco Parolini, Department of Biology, University of Milan, Via Celoria 26, 20133 Milan, Italy.
- Prof. Francois Gagne, St. Lawrence Centre, Environment Canada, 105 McGill, Montreal, Quebec, H2Y 2E7, Canada.
- Ms Kathleen O'Rourke, Shannon Aquatic Toxicity Laboratory, Enterprise Ireland, Shannon, Co. Clare, Ireland.
- Mr Robert Hernan, Shannon Aquatic Toxicity Laboratory, Enterprise Ireland, Shannon, Co. Clare, Ireland.
- Mr Liam Curran, Shannon Aquatic Toxicity Laboratory, Enterprise Ireland, Shannon, Co. Clare, Ireland.
- Prof. Jim Wilson, Zoology Dept, Trinity College Dublin, Ireland.
- Dr Michelle Giltrap, Zoology Dept, Trinity College Dublin, Dublin, Ireland.
- Dr Jenny Ronan, Marine Institute, Oranmore, Co Galway, Ireland.
- Dr Brendan McHugh, Marine Institute, Oranmore, Co Galway, Ireland.
- Dr Iarlaith Connellan, Redbank Hatchery, Co. Clare, Ireland.
- Dr Liam Morrison, Ryan Institute, National University of Ireland Galway, Galway, Ireland.
- Ms Tracy O'Shea, Marine Institute, Oranmore, Co Galway, Ireland.
- Dr Mark Jessopp, Beaufort Research, University College Cork, Cork, Ireland.
- Dr Eugene McCarthy, Marine & Freshwater Research Centre, Galway–Mayo Institute of Technology, Galway, Ireland.
- Dr Pauhla McGrane, Marine & Freshwater Research Centre, Galway–Mayo Institute of Technology, Galway, Ireland.
- Mr John Boyd, Marine & Freshwater Research Centre, Galway–Mayo Institute of Technology, Galway, Ireland.

# Appendix 3 Selected Outputs of Project

## Publications


Quinn, B., Murphy, F., McGann, O. and Foley, V. Assessment of background steroid levels in the marine blue mussel (*Mytilus* spp.) over a 12 month period, measured using human diagnostic techniques. *Submitted for publication* in Chemosphere.

McEneff, G., Barron, L., Kelleher, B., Paull, B. and Quinn, B., 2014. A year-long study of the spatial occurrence and relative distribution of pharmaceutical residues in sewage effluent, receiving waters and marine bivalves. *Science of the Total Environment* **476–477:** 317–326.

Schmidt, W., Rainville, L.-C., McEneff, G., Sheehan, D. and Quinn, B., 2014. A proteomic evaluation of the effects of the pharmaceuticals diclofenac and gemfibrozil on marine mussels (*Mytilus* spp.): evidence for chronic sublethal effects on stress-response proteins. *Drug Testing and Analysis* **6:** 210–219.

McEneff, G., Barron, L., Kelleher, B., Paull, B. and Quinn, B., 2013. The determination of pharmaceutical residues in cooked and uncooked marine bivalves using pressurised liquid extraction, solid-phase extraction and liquid chromatography–tandem mass spectrometry. *Analytical and Bioanalytical Chemistry* **405(29):** 9509–9521.

Schmidt, W., Power, E. and Quinn, B., 2013. Seasonal variations in biomarker responses in the marine blue mussel (*Mytilus spp.*). *Marine Pollution Bulletin* **74:** 50–55.

Quinn, B., Gagne, F. and Blaise, C., 2012. *Hydra*, A model system for environmental studies. *Invited review. International Journal of Developmental Biology* **56:** 613–625.

Quinn, B., Schmidt, W., McEneff, G., Curran, L., O'Rourke, K. and Foley V., 2012. Chronic effects of diclofenac on fish and mussels measured using human diagnostic techniques. Extended abstract, SETAC, Berlin, 2012.

Schmidt, W., O'Shea, T., Quinn, B., 2012. The effect of shore location on biomarker expression in *Mytilus spp* and a comparison to long line mussels. *Marine Environmental Research* **80:** 70–76.

Droguet, M., Devauchelle, N., Pennec, J.P., Quinn, B., Dorange, G., 2012. Cultured heart cells from oyster: an experimental model for the evaluation of marine pollutant toxicity. *Aquatic Living Resources* **25:** 185–194.

Quinn, B., Schmidt, W., O'Rourke, K. and Hernan, R., 2011. Effects of the pharmaceuticals gemfibrozil and diclofenac on biomarker expression in the zebra mussel (*Dreissena polymorpha*) and their comparison with standardised toxicity tests. *Chemosphere* **84:** 657–663.

Schmidt, W., O'Rourke, K., Hernan, R. and Quinn, B., 2011. Effects of the pharmaceuticals gemfibrozil and diclofenac on the marine mussel (*Mytilus* spp.) and their comparison with standardized toxicity tests. *Marine Pollution Bulletin* **62:** 1389–1395.

Parolini, M., Quinn, B., Binelli, A. and Provini, A., 2010. Cytotoxicity assessment of four pharmaceutical compounds on the zebra mussel (*Dreissena polymorpha*) haemocytes, gill and digestive gland primary cell cultures. *Chemosphere* **84:** 91–100.

Barron, L., Nesterenko, E., Hart, K., Power, E., Quinn, B., Kelleher, B. and Paull, B., 2010. Holistic visualisation of the mulitmodal transport and fate of twelve pharmaceuticals in biosolid enriched topsoils. *Analytical & Bioanalytical Chemistry* **397(1):** 287–296.

Quinn, B., Costello, M.J., Dorange, G., Wilson, J.G. and Mothersill, C., 2009. Development of an *in vitro* culture method for cells and tissues from the zebra mussel (*Dreissena polymorpha*). *Cytotechnology* **59:**121–134.


## Planned Publications

Brian Quinn: Background steroid levels in the freshwater zebra mussel (*Dreissena polymorpha*) over a 12 month period, measured using human diagnostic techniques.

Brian Quinn: Effect of 17α-ethynylestradiol on steroid levels in the blue mussel (*Mytilus* spp.) and their comparison with vitellogenin induction measured as ALP.

Brian Quinn: Development and validation of a protocol for the analysis of clinical chemistry endpoints in the hemolymph of the marine mussel *Mytilus* spp. using human diagnostic techniques.

Brian Quinn: Chronic effects of diclofenac on fish and marine mussels (*Mytilus* spp.) measured using human diagnostic techniques.

Brian Quinn: Assessing the biological impact of a 12 month in situ exposure of marine blue mussels (*Mytilus* spp.) to municipal effluent using biomarker and human diagnostic endpoints.

Brian Quinn: Impact assessment of municipal effluent on

wild blue mussels (*Mytilus* spp.) using biomarker and human diagnostic endpoints.

## PhD Thesis Submissions

Gillian McEneff. (January, 2014). *LC-MS/MS Analysis of Pharmaceuticals in the Irish Aquatic Environment and the Potential for Human Exposure*. Dublin City University, Dublin, Ireland.

Wiebke Schmidt. (November, 2012). *Assessment of the Ecotoxicological Effects of Novel Contaminants in the Irish Aquatic Environment on Marine Bivalves, Using a Biomarker/Proteomic Approach*. Galway–Mayo Institute of Technology, Galway, Ireland.

## Platform Presentations

Quinn, B., Schmidt, W., Sheehan, D., McEneff, G., Barron, L., Kelleher, B. and Paull, B. Review of a five year study on pharmaceuticals in the Irish aquatic environment. 23rd Annual SETAC Conference, Glasgow, Scotland. 12–16 May 2013.

Schmidt, W., Evaluation of effects of the pharmaceuticals diclofenac and gemfibrozil on marine mussels (*Mytilus* spp.). Evidence for chronic sublethal effects on stress-response proteins. 17th PRIMO Meeting, Faro, Portugal. 5–8 May 2013.

McEneff, G., Barron, L., Kelleher, B., Paull, B. and Quinn, B. LC-MS/MS analysis of pharmaceuticals in the aquatic environment. Irish Mass Spectrometry Society (IMSS) Conference, Red Cow Hotel Dublin, Ireland. 1 May 2013.

Schmidt, W., Evaluation of effects of the pharmaceuticals diclofenac and gemfibrozil on marine mussels (*Mytilus* spp.). Evidence for chronic sublethal effects on stress-response proteins. 3rd Young Environmental Science Meeting, Krakow, Poland. 11–13 February 2013.

Quinn, B., Schmidt, W., Sheehan, D., McEneff, G., Barron, L., Kelleher, B. and Paull, B. A review of a five year study on pharmaceuticals in the Irish aquatic environment. 23rd ESAI Annual Colloquium, NUIG, Galway, Ireland. 30 January–1 February 2013.

McEneff, G., Barron, L., Kelleher, B., Paull, B. and Quinn, B. LC-MS/MS analysis of pharmaceuticals in the aquatic environment. Invited speaker at the Royal Society of Chemistry Advances in Clinical Analysis, Guys Hospital, London, United Kingdom. 30 October 2012.

Quinn, B., McGann, O., Wilson, J., Giltrap, M. and Foley, V. The assessment of endocrine disruption in the environment using human diagnostic immunoassay techniques. *Invited speaker* at Sino–European Symposium on Environment and Health (SESEH), NUIG, Galway, Ireland. 20–25 August 2012.

Quinn, B., Schmidt, W., McEneff, G., Curran, L., O'Rourke, K. and Foley, V. Chronic effects of diclofenac on fish and mussels measured using human diagnostic techniques. 6th SETAC World Congress, Berlin, Germany. 20–24 May 2012.

McEneff, G., Barron, L., Kelleher, B., Paull, B. and Quinn, B. LC-MS/MS analysis of pharmaceuticals in the aquatic environment. 22nd Irish Environmental Researchers Colloquium, University College Dublin, Dublin, Ireland. 7–9 March 2012.

Quinn, B. and Parolini, M. Development and use of in vitro methods for investigating the acute & chronic toxicity of emerging contaminants (and their mixtures) on the freshwater bivalve *Dreissena polymorpha*. NATO SfP 982590 project workshop: 'Characterization of hazardous chemical contamination – from environmental chemistry and toxicology to risk assessment' (http://www.irb.hr/nato-savariver/), Dubrovnik, Croatia. 23–26 September 2010.

Schmidt, W., Power, E., O'Rourke, K., Hernan, R. and Quinn, B. Chronic effects of the pharmaceuticals gemfibrozil and diclofenac on zebra mussel (*Dreissena polymorpha*) and comparison with standard toxicity tests. 20th ESAI Annual Colloquium. Limerick Institute of Technology, Limerick, Ireland. 17–19 February 2010.

Quinn, B. Biomarkers & bioassays in ecotoxicology & environmental monitoring. 20th ESAI Annual Colloquium, Limerick Institute of Technology, Limerick, Ireland. 17–19 February 2010.

Quinn, B. Human health and environmental effects of exposure to pharmaceuticals released into the environment. Flash Presentation for FP7 Call ENV.2010.1.2.2-2, Brussels, Belgium. May 2009.

Quinn, B. The potential threat of pharmaceutical products to the aquatic environment. ESAI IWWE Conference, RDS, Dublin, Ireland. 25 March 2009.

Quinn, B. Tackling novel contaminants in the Irish aquatic environment: pharmaceuticals, cause for concern? Plenary talk at EPA Scholarship & Fellowship Seminar, Dublin, Ireland. 13 and 14 November 2008.

Quinn, B. 2008. Ecotoxicology and its role in the Water Framework Directive (WFD). Irish Water Waste & Environment (IWWE) Conference, RDS Simmonscourt, Dublin, Ireland. 6 March 2008. (*Invited speaker. Attended by academia, regulation & industry.*)

Quinn, B. The investigation of pharmaceuticals and endocrine disrupting compounds in the Irish aquatic environment: A biomarker approach. 18th ESAI Annual Colloquium. Dundalk Institute of Technology, 2 February 2008.

## Poster Presentations

McEneff, G., Barron, L., Kelleher, B., Paull, B. and Quinn, B. LC-MS/MS analysis of pharmaceuticals in the Irish aquatic environment. 39th International Symposium of High Performance Liquid Chromatography and Related Techniques (HPLC), RAI Conference Centre Amsterdam, Netherlands. 16–20 June 2013.

Schmidt, W., Rainville, L.C., McEneff, G., Sheehan, D. and Quinn, B. Exposure of caged mussels to the effluent of wastewater treatment plants in Ireland. 23rd Annual SETAC Meeting, Glasgow, Scotland. 12–16 May 2013.

Quinn, B., McGann, O., Giltrap, M., Wilson, J. and Foley, V. A novel approach to assess endocrine disruption in the environment using human diagnostic immunoassay techniques. SETAC Europe Environmental Endocrine Disrupter Testing and Evaluation Symposium, Brussels, Belgium. 24–25 October 2012.

Quinn, B., Schmidt, W., McEneff, G., Curran, L., O'Rourke, K. and Foley, V. Using human diagnostic technologies for environmental monitoring; Diclofenac, a case study. EPA STRIVE Research Conference, Trinity College Dublin, Dublin, Ireland. 28 June 2012.

McEneff, G., Schmidt, W., Barron, L., Paull, B., Kelleher, B. and Quinn, B. Solid phase extraction and LC-MS/MS analysis of pharmaceuticals in the Irish aquatic environment. 6th SETAC World Congress, Berlin, Germany. 20–24 May 2012.

Schmidt, W., Rainville, L.C., McEneff, G., Sheehan, D. and Quinn, B. Evaluation of chronic sublethal effects of the pharmaceuticals gemfibrozil and diclofenac on the marine mussel (*Mytilus* spp.) using a proteomic approach. 6th SETAC World Congress, Berlin, Germany. 20–24 May 2012.

Schmidt, W., Rainville, L.C., McEneff, G., Sheehan, D. and Quinn, B. A comparative *in situ* study on the ecotoxicological effects of pharmaceuticals in Ireland, using marine mussels (*Mytilus* spp.). 6th SETAC World Congress, Berlin, Germany, 20–24 May 2012.

McEneff, G., Kelleher, B., Barron, L., Paull, B. and Quinn, B., Solid phase extraction and LC-MS/MS analysis of pharmaceuticals in the Irish aquatic environment. 9th Annual EPA Postgraduate Seminar, Dublin, Ireland. 17 November 2011.

McEneff, G., Schmidt, W., Barron, L., Paull, B., Kelleher, B. and Quinn, B. Solid phase extraction and LC-MS/MS analysis of pharmaceutical and personal care products in the Irish aquatic environment. Instrumental Methods of Analysis (IMA) Conference, Chania, Crete, Greece. 18 –22 September 2011.

Quinn, B. and Parolini, M. Acute and chronic toxicity testing of pharmaceutical compounds on primary cultures from the zebra mussel (*Dreissena polymorpha*). 21st Annual SETAC Meeting, Milan, Italy. 15–19 May 2011.

McEneff, G., Schmidt, W., Barron, L., Paull, B. and Quinn, B. Solid phase extraction and LC-MS/MS analysis of pharmaceutical and personal care products in the Irish aquatic environment and their potential to bioconcentrate. Conference on Analytical Sciences, Ireland (CASi), Dublin City University, Dublin, Ireland. 21–22 February 2011.

McEneff, G., Schmidt, W. and Quinn, B. A comparative *in situ* study on ecotoxicological effects of pharmaceuticals in Ireland. 8th Annual EPA Postgraduate Seminar, Dublin, Ireland. 11 November 2010.

Schmidt, W., McEneff, G. and Quinn, B. Are there chronic sublethal effects in blue mussels when exposed to the pharmaceuticals gemfibrozil and diclofenac? 8th Annual EPA Postgraduate Seminar, Dublin, Ireland. 11 November 2010.

Schmidt, W., O'Rourke, K., Hernan, R. and Quinn, B. Chronic effects of the pharmaceuticals gemfibrozil and diclofenac on the zebra mussel (*Dreissena polymorpha*) and their comparison with toxicity tests. 20th Annual SETAC Meeting, Seville, Spain. 23–27 May 2010.

Quinn, B., Schmidt, W., O'Rourke, K. and Hernan, R. An investigation into the chronic effects of the pharmaceutical diclofenac and gemfibrozil on the marine mussel (*Mytilus edulis*) and their comparison with acute toxicity tests. 20th Annual SETAC Meeting, Seville, Spain. 23–27 May 2010.

Barron, L., Nesterenko, E., Power, E., Quinn, B. and Paull, B. Mass-Balanced investigation of the transport and fate of pharmaceuticals in biosolid enriched topsoils. EuroAnalysis, Innsbruck, Austria. 6–1 September 2009.

Quinn, B., Schmidt, W., Power, E. and Officer, R. The chronic effects of the pharmaceuticals diclofenac and gemfibrozil on biomarker expression in the common mussel (*Mytilus edulis*). International Society for Toxicity Assessment (ISTA), University of Metz, France. 1–4 September 2009.

Power, E., Quinn, B., Paull, B. and Barron, L. 2009. Transport of pharmaceutical residues to the soil environment following biosolid enrichment. 19th Annual SETAC Meeting, Gothenburg, Sweden. 31 May–4 June 2009.

Schmidt, W., Power, E., Robinson, M. and Quinn B. Investigating the chronic effects of the pharmaceuticals diclofenac and gemfibrozil on the common mussel (*Mytilus edulis*) and zebra mussel (*Dreissena polymorpha*). 19th Annual SETAC Meeting, Gothenburg, Sweden. 31 May–4 June 2009.

## Popular Media

*EcoEye*: Brian Quinn interviewed by Duncan Stewart for RTE television. November 2008.

*Sunday Business Post*: Brian Quinn interviewed by Margaret O'Brien for an article entitled *Something in the Water: Key Issues under Discussion*. Printed 15 March 2009.

*Irish Times*: Brian Quinn interviewed by Anthony King for an article entitled *The Undiluted Truth about Chemicals in our Water*. Printed on 5 January 2012.

EPA Research Report 143

# Pharmaceuticals in the Irish Aquatic Environment

epaResearch
Climate - Water - Sustainability
*Identifying pressures • Informing policy • Developing solutions*

Pharmaceuticals are regarded as contaminants of emerging concern and enter the aquatic environment primarily via treated municipal effluent. In this report, a 5-year study was undertaken to:

- Measure the concentrations of five chosen pharmaceuticals in municipal effluents, marine waters and marine biota;
- Assess the potential for human exposure via ingestion of contaminated seafood;
- Investigate the toxic potential of pharmaceuticals to aquatic organisms; and
- Develop protocols and technologies for the identification, quantification and monitoring of pharmaceuticals in the marine environment.

## Identifying Pressures

Ireland has a high level of pharmaceutical consumption, with many of these drugs being insufficiently removed during the treatment of municipal effluents. As the majority of wastewater treatment plants are located on the coast, it is necessary to quantify the levels of these contaminants entering the marine environment, to investigate their toxic potential and the potential for human exposure via contaminated seafood.

## Informing Policy

For the first time pharmaceutical compounds have recently been added to the European Union (EU) Water Framework Directive (WFD) watch list and are currently monitored in EU surface waters. Both the WFD and the Marine Strategy Framework Directive (MSFD) aim to achieve 'Good Environmental Status' in European waters. The combined chemical and biological analysis undertaken in this study provides an integrated approach to assess the fate and potential effects of pharmaceutical pollution in the Irish marine environment.

## Developing Solutions

Marine ecosystems provide ecological, economic and societal services that are under increasing pressure from the threat of pollution. The analytical chemistry techniques and biological assessment, using both established and developing new methods, not only enhance Ireland's capacity towards the integrated monitoring of contaminants but also add valuable baseline data necessary for effective monitoring. During this study, new techniques and protocols were developed to aid in the monitoring of contaminants in the Irish marine environment.

Comhshaol, Pobal agus Rialtas Áitiúil
Environment, Community and Local Government

**EPA Research:** McCumiskey House, Richiew, Clonskeagh, Dublin 14.
**Phone:** 01 268 0100
**Twitter:** @EPAResearchNews
**Email:** research@epa.ie

www.epa.ie
**EPA Research Webpages**
www.epa.ie/researchandeducation/research/