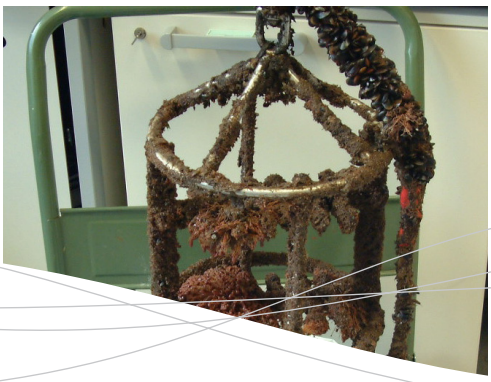
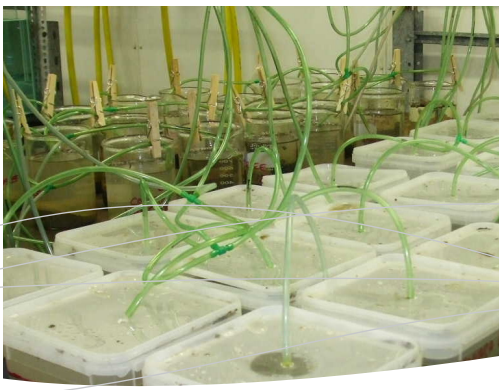
Marine Research Sub-Programme
(NDP 2007-'13) Series

SeaChange
Casadh na Taoide

Biological Effects and Chemical Measurements in Irish Marine Waters

Project Based Award, Final Report





Lead Partner: Trinity College Dublin

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Sea Change: A Marine Knowledge, Research & Innovation Strategy for Ireland

Sea Change—A Marine Knowledge, Research & Innovation Strategy for Ireland 2007-2013—was launched in early 2007 and was the outcome of extensive analysis and consultation with government departments, state agencies, industry and the third-level sector. It outlines a vision for the development of Ireland's marine sector and sets clear objectives aimed at achieving this vision, namely to:

1. Assist existing, and largely indigenous, marine sub-sectors to improve their overall competitiveness and engage in activity that adds value to their outputs by utilising knowledge and technology arising from research.
2. Build new research capacity and capability and utilise fundamental knowledge and technology to create new marine-related commercial opportunities and companies.
3. Inform public policy, governance and regulation by applying the knowledge derived from marine research and monitoring.
4. Increase the marine sector's competitiveness and stimulate the commercialisation of the marine resource in a manner that ensures its sustainability and protects marine biodiversity and ecosystems.
5. Strengthen the economic, social and cultural base of marine dependant regional/rural communities.

The Sea Change strategy was developed as an integral part of the government's Strategy for Science, Technology and Innovation (SSTI) and the Marine Institute as the lead implementation agency is working within SSTI policy and with government departments and agencies to deliver on the Strategy.

The Marine Institute managed Marine Research Sub-Programme, one of eight sub-programmes within the Science, Technology and Innovation (STI) Programme of the National Development Plan 2007—2013, targets funding to meet the objectives of the Sea Change strategy.

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SeaChange Casadh na Taoide

Marine Research Sub-Programme 2007-2013
Project Based Award

Biological Effects and Chemical Measurements in Irish Marine Waters

(Project Reference: PBA-ME-07-001)

Lead Partner:	Trinity College Dublin
Project Partners:	Marine Institute, Shannon Aquatic Toxicity Laboratory, Dublin Institute of Technology
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Project Duration:	01/08/2008 to 31/12/2012

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Executive Summary

The detection of anthropogenic dangerous substances in the environment is a ubiquitous phenomenon with the marine environment subject to inputs from a variety of sources including via polluted rivers, direct discharges and atmospheric deposition. While such contamination tends to be most prevalent closest to the primary sources (e.g. industry, urban, intensive agriculture), remote areas are also subject to pollution, for example by long-range atmospheric transport of airborne substances. Exposure to such complex mixtures of potentially harmful chemical compounds may bring about undesired biological effects including metabolic disorders, increases in disease prevalence, and adverse effects on population growth, effects on reproduction and on the survival of exposed aquatic organisms. This project reports the selection, development, validation and testing of relevant biological effects and contaminant measurement tools for substances of concern in Irish waters. Ultimately the key findings of an integrated assessment at a variety of Irish marine sites are evaluated.

During the course of the project, biomarkers methodologies were established for both "traditional" and "novel" contaminants such as endocrine disrupting compounds (EDCs). Effects of contaminants were investigated using a battery of biomarkers at various organisation levels in a range of organisms. These effects and methodologies were then further supported by an extensive chemical measurement programme in a range of matrices including biota, sediment, water and passive sampling technologies. Overall most biomarker responses were below the associated assessment criteria at many of the sites monitored. In some cases, exceedences of assessment criteria may relate to variability associated with natural background concentrations, may be associated with methodological issues or be reflective of the fact that it is still generally only possible to generate a partial picture of the extent of effects and of the full suite of environmental contaminants present within the environment.

Aggregating the assessments as conservative worst-case scenarios across parameters indicates that few areas exhibit parameters that are flagged with a less-than good (red) status with other than low confidence assigned. The primary areas of concern tend to be estuaries and bays subject to major urban influence. Where trends were measured at relevant test sites they were generally in a downward direction showing an overall improving picture. There is evidence of an improvement with relation to TBT-related imposex effects in *Nucella lapillus* with concerns limited to areas around major fishing/shipping ports.

Clear overlap exists between the European Water Framework Directive (WFD) 2000/60/EC and the Marine Strategy Framework Directive (MSFD) 2008/56/EC. There is a requirement under MSFD indicator 8.2.1 for assessment of pollution effects on ecosystem components at various levels of biological organisation. Given the uncertainty associated with many individual effects techniques and the difficulty for assessment in unambiguously relating them to contaminant pressure the use of such biological effect techniques as "stand alone" indicator/targets to define Good Environmental

Status (GES) (e.g. similar to WFD application of Environmental Quality Standards (EQS) may be problematic.

This project details a limited approach to the use of biological effects tools, in line with the Oslo and Paris Commission (OSPAR) recommended approach, to assess pollution effects. While we do not recommend adding additional specific biological effects as standalone indicators/targets for MSFD purpose, using a greater suite of biological effects in an integrated biological effects-concentrations indicator may give a better overview of ecosystem health. The integrated approach reported provides a method for aggregating across indicators to provide an overall assessment of GES for Descriptor 8 and provides the first quality assured "baseline" information with respect to integration of chemical measurements and biological effects for Irish marine waters. On a spatial scale this project reports that biomarkers/biological effects tools can be used as screening methods to support identification of areas at risk while the full integrated approach is best utilised for assessment of areas at highest risk given the costly nature of such programmes. Biological effects and contaminant data from this project further support the conclusion that, apart from certain specific issues and areas, the quality of Irish transitional and coastal waters is generally good, with some evidence of decreasing levels for certain substances. Ultimately the overall risk to aquatic organisms in general is low.

1. Introduction: Chapter One

1.1. Background

The production and use of chemicals inevitably leads to contamination of the marine environment. Most man-made and naturally occurring substances, some of which are hazardous, are released and may enter the marine environment via a number of sources including direct discharges, such as municipal and industrial waste water treatment plant effluent (WWTPE), agricultural and terrestrial run off, airbourne sources or as a result of accidental release or as losses during the life cycle of products (OSPAR, 2010). Current monitoring and assessment of the pollution status of the marine environment in Ireland is mainly reliant on chemical measurements of the more "traditional" contaminants including metals, polychlorinated biphenyls, polybrominated diphenyl ethers, organohalogens compounds including pesticides and related products, polycyclic aromatic hydrocarbons and organotins in sediments, water and in "bioindicator" species. It has been recognised that chemical based approaches alone provide very limited information on the pressures on the ecosystem and they may miss occurrence/effects of a compound if not included in the target suite. Additionally they do not always elucidate the actual effects of the pressures on the organisms that reside in the marine environment including synergistic or antagonistic effects of compounds and mixtures as well as natural stressors such as salinity or temperature. Until the commencement of this project very few biological effects data have been available for Irish marine waters to further characterise actual effects on the ecosystem and its components.

The Oslo and Paris Commission (OSPAR) and International Council for the Exploration of the Sea (ICES) have developed guidelines for the completion of an "integrated" approach to quality status reporting incorporating assessments built on a set of chemical and biological effects monitoring tools (ICES, 2012). Biomarkers have been used to monitor effects from contaminants for many years (Cole, 1979). These include biomarkers of toxic effects, which are responses from a range of toxicities and also biomarkers of exposure which are responses due to specific compounds or groups of compounds. The objectives of WFD and MSFD are to protect transitional and coastal ecosystems from chemical pollution. ICES and OSPAR activities provide a basis to complement these objectives. Through this project, capacity was developed to report on the recommended OSPAR/ ICES tools for biological effects thus providing the first such baseline data for Irish coastal waters.

Biomarkers and supporting chemical measurement methodologies were established for both "traditional" contaminants as mentioned above and selected "novel" contaminants, such as endocrine disrupting compounds (EDCs) including steroid estrogens, nonylphenol and octylphenol. Traditional contaminant levels and effects were investigated using a battery of biomarkers at various organization levels in a range of organisms and chemical measurements. Endocrine disrupting compounds were investigated using both standardised techniques including immunoassay, imposex and intersex, but also using more novel biomarkers such as alkali-labile phosphate (ALP), gel electrophoresis and the estrogen receptor mediated luciferase reporter gene system (ER-LUC)

assay. These effects and methodologies are then further supported by an extensive chemical measurement program in a range of matrices including biota, sediment, water and passive sampling technologies.

A two tiered spatial and temporal study of contaminants and their effects was used to generate data for the project. Sites were chosen after consultation with an expert committee based on reflection of a range of contamination in Irish estuaries and coastal waters. An initial screening of 9 sites with two physiological biomarkers of toxic effects in the blue mussel "*Mytilus edulis*" and sediment ecotoxicology was completed. This was then complimented by the completion of an extensive battery of biomarkers and chemical measurements in a range of organisms and matrix types at four specific locations selected for full scale assessment in the final phase of the study. This project allowed for an integrated biological effects/chemical assessment for selected test and reference sites in Irish waters through trialling chemical/biological response indices to support overall classification of pollution pressures and ecological relevance.

1.2. Project Description

1.2.1. Project Objectives

The overall aim was to increase Ireland's capacity for the generation of integrated monitoring of biological effects and chemical measurement data and for the completion of a pilot scale assessment of the quality of the Irish marine environment at a number of selected locations.

The major project objectives were to:

1) Set up and validate a suite of quality assured (Biological Effects Quality Assurance in Monitoring: BEQUALM) or equivalent QA standards where appropriate and establish biological effect tools (biomarkers, bioassays),fish disease/pathology and benthic community analyses indices.
2) Concentrate research aspects on optimising developed techniques for the measurement of endocrine disrupting effects, particularly in invertebrates.
3) Develop supporting chemical analysis methodologies primarily concentrating on determination of EDCs in the Irish marine environment and to develop novel techniques such as passive sampling for the detection of these contaminants in the environment.
4) Identify key locations/test sites for the piloting of the proposed test battery of biological effects, benthic and chemical monitoring in order to provide "baseline" assessment data of levels of key suites of contaminants and their associated biological effects at these sites, ultimately linking with ongoing monitoring such as that required under the WFD and MSFD.
5) Develop approaches in line with international recommendations for the integration of biological effects and chemical test data for use in an initial integrated assessment.
6) Report on the achievements of the project.
7) Disseminate assessment information to appropriate agencies such as the Environmental

Protection Agency (EPA), OSPAR and ICES and to peer-reviewed publications and reporting of a number of case studies.

1.2.2. Partners and Associates

Project Coordinator and Lead Partner: Trinity College Dublin (TCD)
Trinity College Dublin's role was to develop a range of biomarkers at various organization levels for a range of organisms. TCD were in charge of the project management including day to day administration, the organization of project steering and external advisory group meetings and the development of a database and project website.

Marine Institute (MI)
The MI Marine chemistry's role was the development of chemical methodologies for a range of EDCs and also for the analysis of the "traditional" contaminants as previously discussed. Data generated from this project will be reported to the MI and ultimately delivered to the ICES database. The Marine Institute possess extensive state-of-the-art marine chemical laboratories with sensitive analytical instruments which were used for analysis of substances for this project. The MI Fish Health Unit (FHU) which specializes in histopathology of fish, routinely carry out histological, virological, bacteriological and antibiotic residues screening, as well as post-mortem examinations. The FHU's role was to provide expertise for histopathology of fish for the project. The MI Benthic Monitoring Unit provided the expertise for benthic analysis and biotic indices calculations.

Shannon Aquatic Toxicity Laboratory (SATL):
The role of the Shannon Aquatic Toxicity Laboratory was to perform sediment ecotoxicology tests for a range of sediment matrices and on a range of organisms at different trophic levels. In addition to this SATL provided facilities and expertise for the exposures of test compounds on aquatic organisms. SATL also provided base/facilities for sampling of sites on the Shannon.

Dublin Institute of Technology (DIT):
The Radiation and Environmental Science Centre (RESC) at DIT provided expertise in DNA damage assays such as the COMET assay.

1.2.3. Collaborations

Centre for Environment, Fisheries and Aquaculture Science (CEFAS)
CEFAS provided training to Irish research scientists for both mussel and fish histopathology. In addition to this, four weeks of ship time included training in sampling methodologies for biological effects analysis and expertise/advice for development of biological effects techniques. CEFAS also provided services including analyses of fish samples for 7-Ethoxyresorufin O-deethylase (EROD), polycyclic aromatic hydrocarbon (PAH) bile metabolites and vitellogenin (V).

1.2.4. Description of the Work Packages

The work programme is divided into five main work packages (WP) as outlined in Figure 1.1 below.

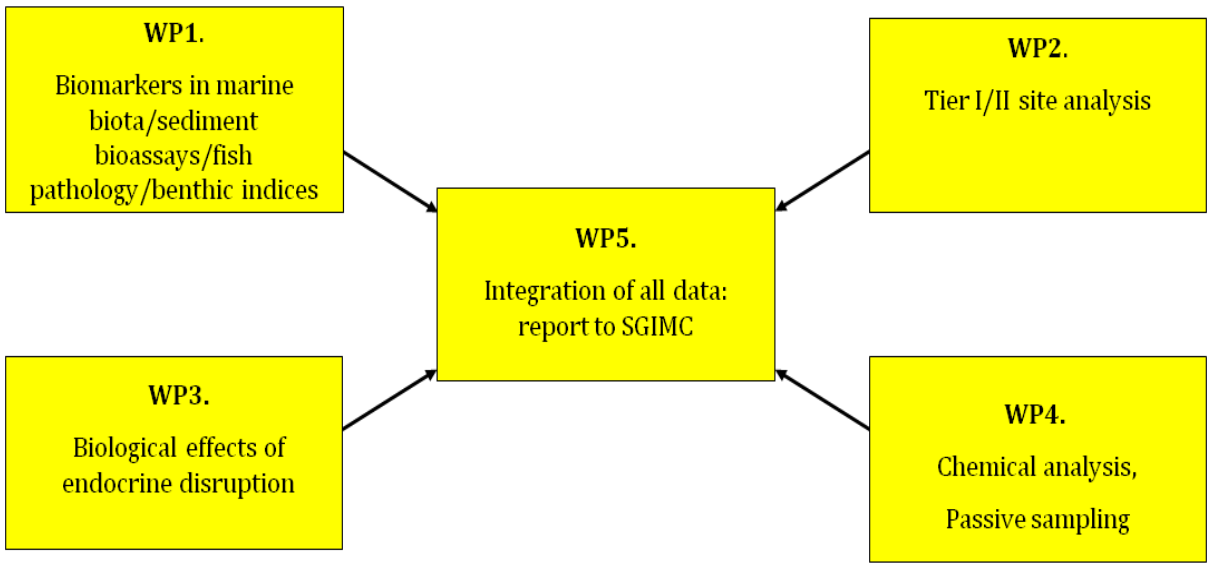


Figure 1.1: Work package descriptions for Sea Change project.

It is recognized that an integrated approach including both chemical measurements and biological effects is required for monitoring of hazardous substances. OSPAR and ICES have increased their focus on integrative assessments and recently produced integrated guidelines. Measurement of biochemical markers or 'biomarkers' (biochemical and /or physiological changes in organisms exposed to contaminants) in individual organisms *in situ* can provide sensitive and specific early warning signs of biological stress in response to pollution. In contrast, measurements at a broader ecosystem scale may be insufficiently sensitive or unable to discern contributory cause-effect relationships. Any suite of monitoring techniques must span the range of ecological complexity from sub-organism level to populations and ecosystems. This project proposed a range of "integrated" chemical and biological effects techniques as candidate biomarkers and bioassays, which cover the range of bio-complexity and which in addition, offer the potential by which specific contaminants can be identified (See Figure 1.2).

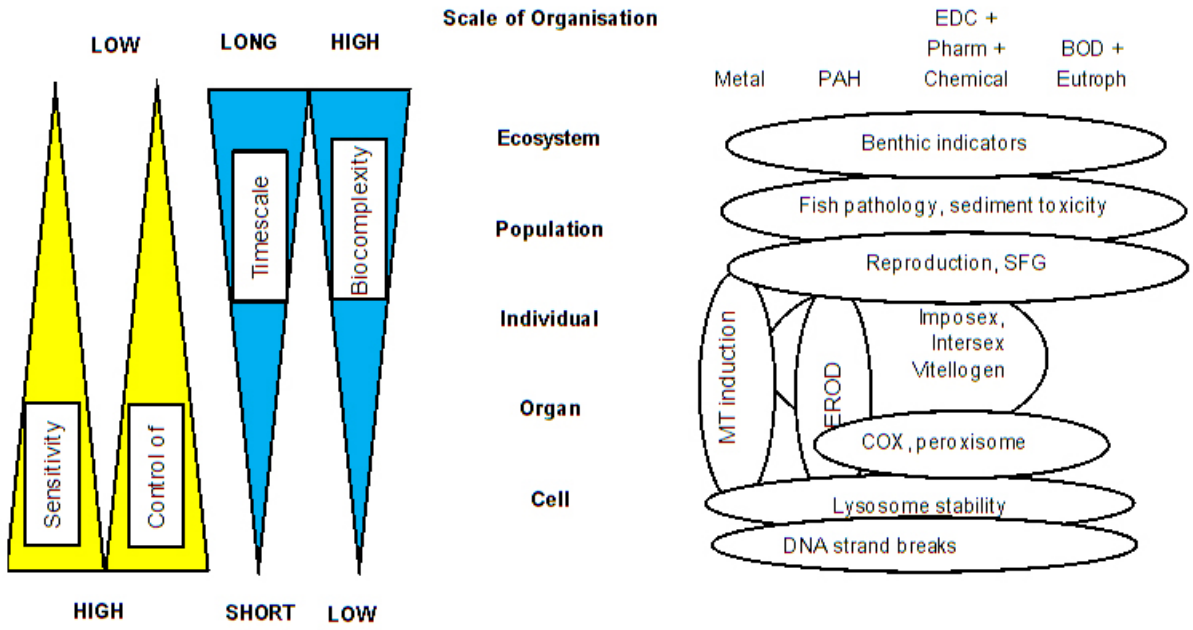


Figure 1.2: Coverage of system and contaminant spectrum by selected biomarkers.

In international monitoring programmes the use of a battery of biomarkers is strongly recommended as an integral part for monitoring of traditional contaminants. In this project early warning biological effect assays (methallothionein (MT), EROD, acetylcholinesterase (AChE), PAH metabolites), biomarkers of damage (DNA strand breaks, Lysosomal membrane stability) and biomarkers of reproduction (Vtg, vitellin-like proteins, ER-LUC, imposex and intersex) are utilised along with scope for growth (SFG), stress on stress (SoS) and histopathology in mussels and a fish disease index incorporating fish histopathology. Sediment toxicity was evaluated using a suite of standardised bioassays and water, sediment and biological samples were chemically analysed for contaminants.

Despite monitoring for the suites of contaminants as detailed under the OSPAR Co-ordinated Environmental Monitoring Programme (CEMP) there is limited routine monitoring for emerging or novel pollutants such as endocrine disrupting compounds such as EDCs undertaken in Ireland. In recent years extensive research, particularly in imposex and intersex on gastropod molluscs and more recently in the feminization of male fish exposed to (xeno) estrogenic compounds has been completed (Sumpter, 1998). Although there is little doubting the feminizing of male fish, the application of the technique in species resident in Irish waters is limited. As such this project aimed to provide valuable "baseline" information of EDC occurrence and effects at selected locations.

There is still limited understanding and application of the complex fate and effect interactions of multiple stressors in assessment of the health status of marine ecosystems. This project aimed to concentrate on the development of a test battery of biological effects and chemical measurement

methodologies and ultimately on the "integration" of derived data. The project investigated the potential for the application of an assessment approach recommended by OSPAR and ICES.

1.2.5. Overall Tiered Approach and Aims

Comprehensive assessment of environmental quality requires multiple biomarker/multiphase exposure testing schemes. A two tiered approach was used for the assessment of nine locations around the coast of Ireland which are representative of a range of contaminant burdens.

Tier I sites were screened with two biomarkers using the marine mussel, *Mytilus edulis*, namely the physiological markers SFG and SoS as well as the supporting parameter, condition factor. Sediment toxicity was evaluated at each of the Tier I sites with a range of toxicity bioassays. Sites at which biological effects and sediment toxicity were observed in Tier I screening were assessed in more depth in Tier II screening with a more extensive suite of matrices and methodologies completed.

Tier II testing involved the use of multiple biomarkers in mussels and fish including metallothionein, AChE activity, alkali labile phosphate, Vtg, EROD, bile metabolites and assessment of DNA damage using the COMET assay. Chemical tests involving the analysis of a suite of organic and inorganic pollutants in tissue, sediment and passive sampling devices were also performed on Tier II sites. Fish external disease and liver histopathology as well as benthic ecology techniques were assessed as a top level indicator of ecosystem health in addition to this. Chapter 2 describes the integration of these biological and chemical measurements to allow visualisation of potential problem and non-problem areas.

2. Monitoring tools for assessment of pollution status in marine waters: Chapter Two

2.1. Biological Effects Techniques in Mussels, Fish and Gastropods

2.1.1. Scope for Growth (SFG)

Scope for growth involves the combination of measurements of chemical contaminants in the tissues of mussels (*Mytilus edulis*) and the physiological measurements respiration, clearance rate and food absorption efficiency (Widdows et al. 2002). Growth is one of the most sensitive measures of environmental stress as it integrates the major physiological responses involving the balance between processes of energy acquisition (feeding and digestion) and energy expenditure (metabolism and excretion). This measurement has been correlated with concentrations of toxic contaminants such as TBT in the tissues of mussels (Widdows and Page, 1993).

2.1.2. Stress on Stress (SoS)

Stress on stress is a measure of the ability of an organism to survive in anoxic/aerial conditions. Field and laboratory studies have demonstrated this technique as a sensitive response to a range of contaminants including heavy metals, organometals and organics (Viarengo et al., 1995; Eertman et al., 1993; Smaal et al., 1991; Hellou and Law, 2003). The LT_{50} measured (time at which 50 % of the animals have died) has been found to be comparable with indices at the cellular level (Viarengo et al. 1995). Early LT_{50}'s can potentially indicate pollution induced alterations in the organisms physiology that can render the organism more sensitive to further environmental changes and possibly contaminant burden.

2.1.3. Lysosomal Membrane Stability (LMS)

The lysosomal system provides a waste removal and macromolecular recycling system and also functions as a membrane bound compartment for intracellular digestion of food ingested by cells. As such it has been identified as a target for the toxic effects of many contaminants. Lysosomes encompass a detoxification capacity by accumulating many toxic metals and organic chemical contaminants, which if overloaded results in lysosomal damage leading to cell injury, tissue disfunction, and therefore reduction in animal health status. Indicators of cell reactions to pollution include loss of membrane integrity, enlargement, and accumulation of lipid and lipofuscin (Moore et al., 2004). The neutral red retention (NRR) technique for blood/haemolymph cell lysosomes measures the efflux of the lysosomal contents into the cytosol, thereby reflecting, in the case of impacted mussels, a normal physiological process that has become compromised following damage to the membrane (Lowe et al., 1995).

2.1.4. Metallothionein (MT)

Metallothioneins are low molecular weight (6-8 kDa), cysteine-rich (20-30%), metal-binding proteins found in all vertebrates and invertebrates whose synthesis represents a specific response to pollution of heavy metals (Viarengo, 1989). These proteins can bind essential (copper, zinc) and also non-essential (cadmium, mercury) metal cations in a non-toxic form, thus reducing their adverse effects (Viarengo and Nott, 1993). Metallothionein has been determined to be a sensitive biomarker of metal contamination but it can also be induced by other environmental stressors that trigger inflammation and oxidative stress (Stegmann et al., 1992). A low cost spectrophotometric sulfhydryl method for measurement of MT in tissues of marine organisms was developed by Viarengo et al. (1997) and is used in this project.

2.1.5. Acetylcholinesterase (AChE) Assay

Organochlorine pesticide use has been restricted in recent years due to the environmental persistence of these contaminants. Some of these contaminants have been determined to be neurotoxic including organophosphate and carbamate insecticides. These contaminants can block the breakdown of acetylcholine (ACh) by the enzyme, acetylcholinesterase (AChE). This can result in a build up of ACh causing over-stimulation of sensitive neurons at the neuromuscular junction and can lead to spasm and tremors in the organism. Methods for biomonitoring are established for muscle and brain tissue of fish and for gill tissue or haemolymph in mussels (Kirby et al., 2000; Bocquené and Galgani, 1998).

2.1.6. Vitellogenin and Vitellin like Proteins Alkali Labile Phosphates (ALP)

Endocrine disrupters are exogenous compounds that act like hormones in the endocrine system of organisms and disrupt the physiological function of endogenous hormones. Pesticides, polycyclic aromatic hydrocarbons, certain polychlorinated biphenyls, dioxins, furans, alkylphenols and steroids can be considered EDCs (Depledge and Billinghurst, 1999; Norrgren et al., 1999). Xenoestrogens can act as an estrogen antagonist, binding to the estrogen receptor and eliciting a biological response (Jobling et al., 1996, Quinn et al., 2004). The natural and synthetic steroid estrogens (e.g. 17β-estradiol and 17α-ethinylestradiol) have the highest potencies (Desbrow et al., 1998; Streck, 2009). Pathways to the marine environment for these compounds include via municipal effluents and they can cause various biological effects on both vertebrate and invertebrate species, e.g. feminisation of exposed males or changes in male fecundity and testicular growth (Vos et al., 2000). Increased levels of the egg yolk precursor protein, Vtg in the plasma of male fish and elevated levels of vitellin-like (Vn-like) protein in mussels (Quinn et al., 2004, Blaise et al, 1999) can both be used as biomarkers of endocrine disruption.

2.1.7. COMET Assay

Exposure of genotoxic compounds has been previously assessed by analysis of damaged DNA. Exposure to compounds such as heavy metals and organic pollutants, e.g. PAHs, can lead to

DNA damage. The COMET assay is a method used to measure DNA lesions that play a role in mutagenesis and initiation of cancer and is therefore used as a biomarker for environmental biomonitoring (Mitchelmore and Chipman, 1998; Cotelle and Férard, 1999). Advantages with the COMET assay are that genotoxic damage can be detected in most eukaryotic cell types at a single cell level, only a small number of cells are required, and it can provide an early warning response to genotoxic exposure.

2.1.8. EROD (7-Ethoxyresorufin)

EROD is one of the key biomarkers to monitor biological effects of contaminants. EROD has been used as a marker of mixed function oxygenase (MFO) system activity. The MFO system activity is of importance in the detoxification of several classes of planar organics, most specifically polychlorinated biphenyls (PCBs) and some polycyclic aromatic hydrocarbons (PAH). EROD activity is induced as a response of exposure to these contaminants (Kirby et al., 2004; Stagg and McIntosh, 1998).

2.1.9. Bile Metabolites

PAH bile metabolites in fish bile have been used as a biomarker of PAH contamination since the early 1980's. Storage of bile in the gall bladder permits a degree of accumulation of metabolites and hence higher concentrations of PAHs if present. The presence of metabolites in bile is the final stage of biotransformation of these compounds after which they are finally passed from the organism in bile or urine (Ariese et al., 2005).

2.1.10. Imposex in Marine Gastropods

Tributyltin (TBT) is an effective anti-fouling agent that has been used in marine paints for shipping and for fish cages. It is toxic to many marine organisms at very low concentrations and can impair reproductive performance in a number of molluscan species (Minchin, 2003). There has been a direct link with TBT and the development of imposex "the imposition of male characteristics on female gastropods" at very low levels in the marine environment and this measurement has been adopted by OSPAR as a sensitive, quantitative and reproducible biomarker, under the OSPAR Joint Assessment and Monitoring Programme (JAMP) guidelines (OSPAR 2008).

2.2. Sediment Ecotoxicology

Sediment quality of any aquatic ecosystem is essential for sustaining the health of that system. Sediments have been recognized as a major repository/sink for persistent toxic contaminants. Sediment pollution can lead to disruptions in marine benthic communities (Pearson and Rosenberg, 1978). Toxicity of sediment is assessed through exposure of organisms to phases of whole sediment, porewater and elutriates. Sensitivity of species can vary in response to different contaminant groups

and therefore the most ideal method for assessment is by using a range of test organisms from various trophic levels. Several bioassays should be used as different toxicants can elicit diverse effects in the same test organism (Macken et al., 2008). This is the recommended methodology by ICES (ICES, 2012) and is the approach taken in this project.

2.3. Benthic Ecology

Macrobenthic communities have been used for many years as indicators of pollution. A major advantage of using macrobenthic communities as indicators of pollution is that they are relatively long lived and spatially stable (Pearson and Rosenberg, 1978). Typically, they are not organisms prone to migration, so are excellent integrators over time of water and sediment quality. These organisms are also an integral part of the food web and the species of which they are composed show different tolerances to pollution stress and disturbance. Macrobenthic community analysis is used as a top level pollution indicator in this project at the four Tier II locations.

2.4. Fish Disease and Fish Liver and Mussel Histopathology

2.4.1. Fish Diseases

Fish diseases have been used as a way of measuring the general health status of fish populations for many years and only in the past decade have certain links been made between diseases and pollution (Watermann and Kranz, 1992). Fish disease provides evidence of definitive biological responses and may be indicative of historical exposure with identification of histological lesions having been used as a sensitive indicator of the health of the individual (Stentiford et al., 2003) with some links of exposure to some xenobiotic compounds and toxicopathic hepatic lesions in fish having been reported (Stentiford et al., 2003). In this project fish liver histopathology is used as a top level indicator of environmental health of dab and plaice.

2.4.2. Mussel Histopathology

Mussel histopathology provides an effective set of tools for the detection and characterisation of toxicopathic pathologies, which are increasingly being used as indicators of environmental stress, in addition to disease. It has been selected as a promising technique (tissue response) for inclusion within the mussel integrated approach for biological effects and chemical monitoring as recommended by OSPAR and ICES (ICES, 2012). It includes a range of health parameters that can be employed in monitoring programmes designed to assess the biological effects of contaminants. Specific pathologies have been previously associated with contaminant exposure and pathogens. Mussel histopathology as a technique serves as an anchor for biomarker response and is used as part of a multi-test approach.

2.5. Chemical Analysis

2.5.1. Analysis of Seawater

The WFD and the EQSs have been developed for the most part for water. Environmental Quality Standards are set for metals in dissolved waters and for other substances in total waters, which includes both the dissolved and particulate bound contaminant concentrations. For many substances of concern for the marine environment, i.e. persistent organic pollutants (POPs), water monitoring presents difficulties as analytical challenges can arise due to salt interference, from contamination or due to the difficulty of detecting many low solubility substances at environmentally relevant concentrations. The variability can be spatially and/or temporally high in tidal waters necessitating increased frequency of sampling which can be prohibitive in sample acquisition and analytical costs. Water analysis within this project was restricted to selected spot samples and to passive sampler analysis for EDCs with polar organic chemical integrative samplers (POCIS) and PAH and selected PCBs by polydimethylsiloxane (PDMS) passive sampling; procedures used are documented throughout.

2.5.2. Application of Biomonitor Species

Current temporal and spatial contaminant monitoring programmes completed by the Marine Institute primarily focus on the use of biomonitor species, such as bivalve molluscs, to act as a proxy indicator of contaminant levels within the water column. This is because in general, any contaminants accumulated by a biomonitor organism represent the bioavailable fraction present in the sampled medium and many of the pollutants may be highly concentrated in the tissues of such organisms. Such tools are widely used and in line with the OSPAR guidelines (OSPAR, 1992) and formed a major component of data reported within this project.

2.5.3. Analysis of Sediments

The ultimate fate of POPs in an aquatic environment is linked to sediments. It is generally accepted that the world's oceans are the final recipients and the ultimate sink for many contaminants. Hence, the analysis of sediments can give a valuable insight into the presence of persistent pollutants in aquatic environments. Inland and coastal waters are subject to long term pollution with waste organic matter from human activities. This organic matter can contain a wide variety of anthropogenic pollutants. Once present in the sediment these contaminants can become a base for transfer of chemicals to benthic biota through ingestion or absorption from sediment particles and the water column. Anthropogenic pollutants deposited in this manner can then biomagnify from benthic species throughout the food web. Sediment monitoring must account for critical co-factors that are strongly associated with contaminant concentrations such as grain size and organic carbon. As such sediment monitoring and assessment comprises a significant part of this assessment.

2.5.4. New Tools for Monitoring

In the absence of reliable instruments for semi-continuous *in situ* measurement of relevant target contaminants in water, passive samplers provide a new approach to monitoring that allows

estimation of "time-integrated" dissolved water concentrations at levels generally well below those that can be achieved using spot sampling techniques. The EC CMA (2009) recognise passive sampling as a promising complementary technique. ICES and OSPAR are also developing tools for integrated chemical and biological effects monitoring to facilitate more robust assessments of marine pollution status. Such "integrated" tool sets involving both chemical monitoring of various matrices and biological effects monitoring are regarded as the way forward for monitoring pollution status of the marine environment (Law et al., 2010). Imposex and intersex in gastropod molluscs are examples of very specific biological effects related to TBT contamination. Sediment toxicity assessments also provides a significant component of this assessment

2.6. Methodology: Integrated Assessment Overall Approach

2.6.1. The Approach

- Review, selection and collation of project data and relevant MI datasets.
- Review and collation of appropriate tools and in particular assessment criteria for classification.
- Assessment process, consisting of:
    i. Data extraction and normalisation where appropriate.
    ii. Classification according to international best practice and in line with OSPAR and ICES recommended approaches.
    iii. Detailing the confidence of this assessment.
    iv. Incorporation of relevant CEMP based temporal trends of various parameters into the assessment.
    v. An expert commentary on the above and considering inter alia data available for substances where assessment criteria could not be identified and other information that sheds light on the pollution status of Irish waters.

The bulk of the data used for this assessment originates from within the MI /EPA funded Sea Change project 'Biological effects and Chemical Measurements for the Assessment of Pollution in Irish Marine Waters'. These are further supported by data generated under specific monitoring programmes and research activities by the Marine Institute and TCD. The following datasets were included in the assessment.

2.6.2. MI Research Data

Chemical measurement data was specifically collected in a range of matrices including fish, mussels and sediment. Analysis of passive samplers enabled the estimation of time integrated dissolved phase water concentrations for POPs, which often cannot be measured directly in water as methods are inadequate for detection at relevant environmental concentrations. Within certain confines passive sampling (PS) undoubtedly provide a very effective tool for cost-effective monitoring of water quality and as a support

to environmental and ecotoxicological assessment. The MI deployed PDMS and POCIS passive samplers at each of the Tier II sites presented in this assessment. Overall data clearly provide a picture of good water quality at test sites and overcomes some limitations of traditional monitoring techniques.

2.6.3. Shellfish Waters Directive

The Marine Institute has for many years routinely monitored trace metals, PCBs and organochlorine pesticides (OCPs) in shellfish flesh from designated shellfish growing waters. Samples are collected by MI officers or by Sea Fisheries Protection Authority (SFPA) officers and analysis of target parameters and co-factors (e.g. moisture and lipid) is carried out at MI laboratories. In order to support spatial aspects of this report analysis completed at sites relevant to the project were included in this assessment.

2.6.4. OSPAR Coordinated Environmental Monitoring Programme (CEMP) (MI)

A number of stations, including those selected as indicative of key pressures (urban, river inputs) and some shellfish growing waters, have been designated as relevant for the purposes of spatial and temporal trend monitoring. Data are collected for trace metals, PCBs, OCPs and, at certain sites, brominated flame retardants (BFRs), PAH and other pollutants. Sediment and biota data are reported to the ICES database and used by OSPAR for convention wide assessments (e.g. temporal trend assessments) and for the development of assessment criteria. As well as providing data to contribute to the compliance assessment, the output of a recent trend assessment using relevant data is included in this report.

2.6.5. Other TCD Research Projects

Through student projects and research technicians, a range of effects measurements and other methodologies were developed in addition to the core project techniques. These included intersex in marine gastropods, ferric reducing ability of plasma (FRAP) assay for analysis of oxidative stress, impact of parasites on biomarker response, age classification and growth rates of fish species, *in vivo* studies of endocrine disruptors, caging studies and histopathology in mussels. Two extra sediment toxicity assays were developed by the SATL in addition to the ones listed in the original proposal.

2.6.6. CEFAS and Other Support

Collaborations with CEFAS allowed training in the field of mussel histopathology, fish histopathology and advice and training for many other biological effects techniques to be undertaken. In addition to this, CEFAS awarded the Sea Change project ship-time on the CEFAS ENDEAVOUR including access to personnel for collection of fish samples in the Irish Sea. This amounted to the equivalent of approximately €100k of extra funding. Optimizing sampling opportunities on cruises of the R.V. *Celtic Voyager* was possible with the Science at Sea program and TCD training program for MSc students. This also allowed project members to collect a greater number of samples including data from additional species than were originally planned in the proposal.

3. Materials and methodology: Chapter three

3.1. Biological Effects Techniques in Mussels, Fish and Gastropods

3.1.1. Sampling

For spatial sampling of sediment and biota, all techniques complied with OSPAR JAMP procedural guidelines (OSPAR 1992) or advice from background documents for biological effects (ICES, 2012). All details of sampling for both biological effects and chemical monitoring for Tier I sites are outlined in Giltrap et al. (2013) with the exception of sediment sampling for bioassays. Subsamples of sediment for toxicity testing were analysed at the SATL following International Organisation for Standardisation (ISO) guidelines for preparation for toxicity testing. All caging study and fish sampling details are outlined in Rochford (2012) and Ronan (2013). For benthic sampling, Marine Institute guidelines were adhered to which were based on a number of publications for approaches in benthic sampling including UK National Marine Monitoring Programme – Green Book: http://www.sepa.org.uk/marine/; Holme and McIntyre (1984); Rumohr (1990); Rees et al. (1991); Davies et al. (2001); Eleftheriou and McIntyre, (2005)For TBT related effects such as imposex, the JAMP guidelines were adhered to (OSPAR, 2008).

3.1.2. Surveys

Tier I sampling involved low tide shoreline sampling. Tier II involved surveys conducted in year two of the project including time on the *Celtic Voyager* (Oct/Nov 2008-2010) and *CEFAS Endeavour* (July 2009/2010) research vessels and other contracted commercial fishing boats. Small craft boats were used for caging studies including deployment of passive samplers.

3.1.3. Scientific Analysis

3.1.3.1 Biological Effects Methodology

The methodology for SFG, SOS, condition factor (CF) and contaminant measurements in *Mytilus edulis* are reported in Giltrap et al (2013). Methodology for AChE in mussel gills and brain and muscle of fish, MT in mussel digestive gland and ALP in mussel gonad are outlined in Rochford, (2012). Acetylcholinesterase assay was performed in accordance with Bocquené and Galgani, (1998). Metallothionein and ALP were based on methodologies outlined in Viarengo et al. (1997) and Blaise et al (1999). The COMET assay method for assessment of DNA damage was adapted from a method by Singh et al. (1988). The LMS assay was performed in accordance with Moore et al. (2004), while samples were tested for EROD activity using fluorometric methods in accordance with ICES (1998). Levels of reported EROD activity were normalised to the protein content of the liver supernatant. Protein content was determined by the Bradford assay (Bradford, 1976). Bile metabolites were tested in accordance with Ariese et al. (2005). Fish external disease and liver histopathology including identification of normal liver and five liver histopathology categories (NNT: non-neoplastic toxicopathic; NSI: non-specific inflammatory; FCA: foci of cellular alteration; BN: benign neoplasm;

and MN: malignant neoplasm) were identified as per Feist et al. (2004) and Stentiford et al. (2009). Fish Vtg measurement in male fish was conducted by CEFAS laboratories in Weymouth, UK, in accordance with Scott and Hylland (2002).

3.1.3.2 Sediment Ecotoxicology Test Methods

Both the amphipod *Corophium volutator* and the polychaete *Arenicola marina* are recommended as part of the ICES integrated approach to assess the acute toxicity of whole marine sediments while the harpactoid copepod Tisbe sp. are recommended for porewater/elutriate tests (ICES, 2012). Sediment phases and a battery of bioassays were selected based on previous Irish data incorporating validated established marine bioassays (Macken et al. 2008). The following test methods were used for assessment of sediment toxicity:

- Acute lethal toxicity to marine copepods (Copepoda, Crustacea) (ISO 14669, 1997)
- Marine algal growth inhibition test (ISO 10253, 2006)
- *Vibrio fischeri* luminescent bacteria test (ISO 11348-3, 2007)
- Amphipod *(Corophium volutator)* sediment bioassay test (ICES, 2001a)
- Polychaete *(Arenicola marina)* sediment bioassay test (ICES, 2001b)

Results are presented as LC_{50}'s or as percent inhibition at the top concentration.

3.1.3.3 Chemical Analysis Methodology

Chemical methodologies for traditional contaminants including metals and organics are outlined in Giltrap et al. (2013). All other methodologies for EDCs and EDC effects are outlined in Ronan (2013).

3.1.3.4 Statistics

All statistics used for data analysis are outlined in Giltrap et al. (2013), Rochford, (2012) and Ronan, (2013). Principal component analysis (PCA) was carried out using the PRIMER 6 and PERMANOVA + package.

3.1.4. Assessment Procedure Methodology

All details for assessment methodology used for integrated assessment including assessment criteria and data treatment methods are outlined in Appendix 2.

3.1.4.1 Classification Procedure and Assessment Criteria

The assessment process involved assessing aggregated data against relevant available assessment criteria to assign an appropriate status. In some instances, although criteria were identified,

there were concerns as to their comparability with the data thus outputs are flagged with lower confidence. Selection of appropriate assessment criteria to best represent the classification boundary is key to the classification process. This report considered available assessment criteria and selected the most appropriate criteria for contaminant concentrations and biological effects in water, biota and sediment to support the classification procedure. Limitations associated with these assessment tools should be understood, such as: criteria may not take into account natural variability and local/ regional conditions; criteria may be applied in a different context than originally intended and there is often limited guidance on how criteria should be used. Differences in matrix and/or differences in the parameter analysis method between the actual criteria and collected monitoring data may affect comparability. With the exception of the "pilot" EDC assessment, chemical and biological effects status is completed in line with OSPAR and ICES guidelines. Details on the assessment approaches used and associated limitations are presented throughout this report and in Appendix 2.

Assessment criteria hierarchy gave precedence to the use of OSPAR Background Assessment Criteria (BACs) and Environmental Assessment Criteria (EACs) as prepared for both chemical monitoring and biological effects assessment. In the wider context OSPAR has attempted to derive EACs for biota and sediment in accordance with WFD Annex V methodology. However in many instances the EACs derived were impractical in that they were well below estimated natural "background" concentrations. For this reason OSPAR had to identify alternatives to EAC for assessing CEMP data which were used in the Quality Status Report 2010. In the case of EDCs EC EQS as stipulated in Directive 2008/105/EC and EQS for relevant pollutants as set out in SI 272 of 2009 were utilised where relevant and were further complemented by literature and peer review derived assessment criteria where possible. The assessment criteria for water, biota and sediment used for this analysis are listed in Appendix 2 and associated issues including confidence assessment are further elaborated throughout. These criteria were either used to assess compliance of mean or maximum concentrations. In general mean or maximum concentrations that exceed the appropriate assessment criteria result in red classification indicating less than good determination for that parameter/matrix combination while those that comply are given a blue/green classification (good). This report has endeavoured to use the most appropriate assessment criteria for this assessment. Where there are particular issues such as recognised limitations in using ERLs or EACs or instances where exceedances may be due to underlying local geology and/or natural variability data are classified appropriately.

3.1.4.2 Treatment of Limits of Quantification (LoQs)

Supporting temporal contaminant data used in this assessment were collected in accordance with OSPAR CEMP protocols and were assessed as per procedures used for the QSR (2010). Spatial contaminant data including EDC data collected within this project were compiled in accordance with OSPAR protocols. However, where data were recorded at less than the limit of quantification for the method the mean upper bound value was directly assessed against the relevant assessment criteria.

3.1.4.3 Quality Assurance of Data

Where project partners completed testing in-house, these were successfully carried out in accordance with best practice for marine monitoring. Quality criteria were set such that data were suitable for onward reporting to the ICES database. The use of laboratory and certified reference materials, procedural blanks and duplicate analyses underpinned the testing regime. MI is accredited to ISO17025 for many parameters and routinely participates in proficiency testing such as QUASIMEME (Quality Assurance for Marine Environmental Monitoring in Europe) and AQUACHECK. Trinity College Dublin and SATL successfully participated in BEQUALM programmes where relevant. Where testing was subcontracted, partners made every effort to ensure appropriate experience and quality assurance was in place.

3.1.4.4 Assessment of Temporal Trends

OSPAR temporal trend programmes are continuous programmes designed to detect long-term trends in concentrations or effects of substances in the maritime area with time series data assessed for linear and non-linear trends over selected time intervals using agreed methodologies. Temporal assessment data included in this document are based on samples collected in 2010 with long term trends in some cases generated based on data collected from 1998-2010. The assessment approach is as per that completed by OSPAR for the QSR, 2010 and in accordance with Fryer and Nicholson (1999).

Concentrations of contaminants in biota (mussels, oysters and fish) were compiled on a dry weight basis while metals and organic compounds in sediment were normalised to 5% aluminium concentrations and 2.5% organic carbon, respectively. Performance in internal and external QC exercises are used to support data confidence and significance levels for assessments and the power of the data series at each station to detect changes in concentration was established. For each time series, the normalised concentrations and their uncertainties were used to construct annual contaminant indices, which were then assessed for trends. Reported data were normalised to an appropriate basis and to a relevant co-factor. Median log-concentrations in the last year of monitoring were assessed for trends as follows:

- 7 or more years of data: a smoother was fitted to the median log-concentrations
- 5-6 years of data: a linear trend was fitted
- 3-4 years: a mean is fitted
- 1-2 years: the maximum value was used for graphical purposes only (no statistical tests completed.

The direction of the individual temporal trends is then indicated using arrows. Examples of temporal trend plots supporting the assessment are presented in Appendix 3.

3.1.4.5 Confidence in Assessment and Risks of Misclassification

A risk of mis-classification of an individual sample (false good or false less than good status) can arise due to inadequate available analytical methodology, contamination during sampling (especially for seawater), natural variation of the population (especially for MAC-QS where the upper end of the distribution is being assessed), spatial/geological variability in background concentrations, and insufficient sampling/data. Information on confidence in the assessment output is reported in Appendix 2 and detailed throughout the report. Assessment outputs are colour coded with a striped pattern to indicate low confidence which may be a consequence of insufficient data, inappropriate criteria or other factors.

3.1.5. Sampling Maps

Site selection was completed in order to cover a number of marine water body types and pressure loadings. Additionally criteria for site selection included suitability for deployment of longer–term monitoring equipment (passive samplers, temperature probes etc.) and suitability for use in future temporal monitoring capacity. It must be noted that the primary objective of this project was to further develop technical capacity for the completion of "integrated" chemical and biological effects monitoring and to complete "pilot-scale" integrated assessments using conserved datasets. As such, only limited sampling and analysis was completed. Full spatial sampling coverage of these large bays and estuaries was not possible within the constraints of the project therefore resolution of reported data is spatially restricted and extrapolation of the assessment classification beyond the sampling points should not be undertaken.

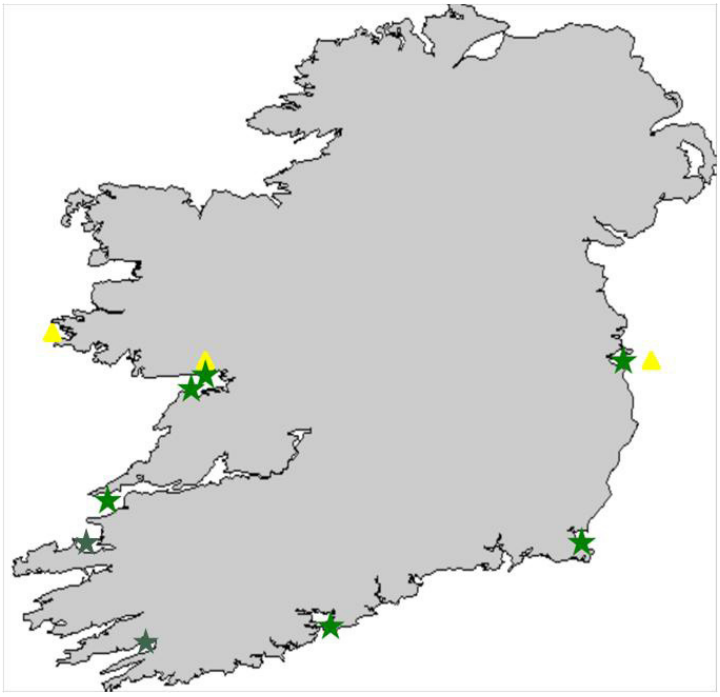


Figure 3.1: Tier I and II sampling locations. Tier I sites are denoted with green stars i.e. Dublin Bay, Wexford Harbour, Cork Harbour, Bantry Bay, Tralee Bay, Kinvarra Bay, Newquay, Galway Bay (Lettermullen) and Shannon estuary. Caging study sites (denoted with yellow triangles): Dublin Bay (North Bank Lighthouse), Galway Bay (Mutton Island) and Omey Island.

4. Tier I and II studies, case studies and additional studies: Chapter four

4.1. Biological Effects Techniques in Mussels, Fish and Gastropods

4.1.1. Tier I Results

A tiered approach for biomonitoring whereby a screening process of a large number of sites with one or two biomarkers is followed by a complete set of biological and chemical analyses at a smaller number of test locations has been recommended by Viarengo et al., (2007). Tier I site selection was primarily based on a review of available chemical and biological data and potential contaminant influences and pressures at a number of Irish coastal locations. It was also based on practicality of access for sampling and on potential use in future monitoring programs. Historical data and potential pressures for the nine sites chosen are detailed in Appendix 4. Tier I studies were performed at nine locations using the early warning biomarkers SFG including CR, RR, and SoS with the supporting parameter condition factor (CF). In addition to this, toxicity studies on sediment matrices were performed as described in the methodology section. It was not possible to use LMS as a Tier I screening biomarker at the same time as SFG due to effects on the response of this biomarker at this time of year. To address these concerns a supplementary case study on the most effective time of year to use the LMS biomarker was completed and is described in section 5.4.

4.1.2. Overall Outcomes from Tier I

4.1.2.1 Biomarker and Contaminant Analysis in *Mytilus edulis*

Both SFG and SOS at the level of the individual in *Mytilus edulis* were evaluated against contaminant levels at sites around Ireland. The sites chosen ranged from moderate to low pollution levels on a European scale (Appendix 4), but the actual ranking of the sites varied according to which contaminants were chosen for the ranking (see Table 4.1 for contaminant concentrations).

At Cork, 4 out of 15 contaminants assessed exceeded the EAC, while at Shannon, no EACs were exceeded. In general, most sites had levels of contaminants below the EAC. EACs for PCBs were exceeded at Cork, Dublin and Bantry including an exceedance for PCB118 at all three sites, however it must be noted that this contaminant has a very low EAC (McGovern et al., 2011) thus this may reflect residual environmental pollution associated with diffuse historical sources and atmospheric inputs as well as environmental variability and may not be due to local or recent inputs of PCBs. All PAHs were below EAC and only lead (Pb) at a known historically elevated location in Cork (Ringaskiddy) was elevated in comparison to the EC food value. Exceeding EC food safety based criteria are not indicative of potential adverse effects and these exceedences should be taken with caution when used for environment risk purposes. While a range of other contaminants were measured in *M. edulis* these were excluded for the initial Tier 1 assessment, all available data are

however included in the overall "integrated" assessment. Principal components analysis revealed that Bantry separated furthest from all of the other Tier 1 locations (see Figure 4.1).

Table 4.1: CEMP contaminants (µg kg^{-1} dry weight) in *Mytilus edulis* at Tier 1 locations around the Irish coast with relevant assessment criteria applied output. Colour key: <BAC-Blue, >BAC<EAC/EC-Green, >EAC/EC -Red as per relevant assessment criteria.

	Shannon	New Quay	Tolka	Tralee	Cork	Kinvara	Bantry	Wexford
PCB 28	0.03	0.19	2.63	0.17	21.7	0.14	0.70	0.16
PCB 52	0.14	0.29	3.51	4.09	15.5	0.14	1.94	0.16
PCB 101	0.18	0.20	15.1	2.88	NA	0.11	1.66	0.67
PCB 118	0.19	0.15	19.9	0.43	23.2	0.20	2.57	0.76
PCB 138	0.36	0.38	12.4	0.68	14.5	0.24	2.57	0.91
PCB 153	0.31	0.21	14.3	1.11	15.2	0.65	12.6	1.54
PCB 180	0.09	0.19	0.64	0.17	0.4	0.16	7.62	0.16
Benzo(a)anthracene	<1.37	2.86	3.1	3.27	2.59	NA	3.27	3.14
Benzo(a)pyrene	<1.37	2.86	3.1	3.27	2.59	NA	3.27	3.14
Benzo(ghi)perylene	<1.37	2.86	3.1	3.27	2.59	NA	3.27	3.14
Fluoranthene	<1.92	4.00	4.3	4.58	3.63	NA	4.58	4.40
Phenanthrene	2.70	5.70	6	6.50	5.20	NA	6.50	6.30
Pyrene	1.37	2.86	3.1	3.27	2.59	NA	3.27	3.14
Cd	939	657	1032	2030	795	727	735	593
Hg	191	130	165	166	170	196	114	120
Pb	2098	1849	8631	735	21982	1738	7307	1550

NA:Not analysed

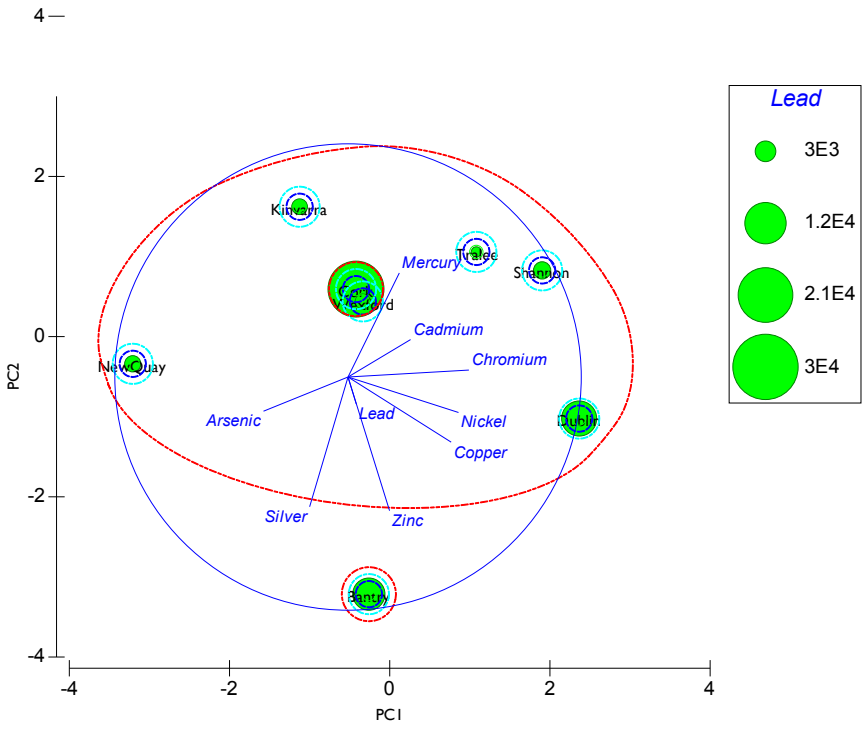


Figure 4.1: Principal components analysis of heavy metals in *Mytilus edulis* sampled from nine Tier 1 locations

In contradiction to the chemical analysis results, the SOS results suggested that Cork was the healthiest site with the longest LT_{50} of 17.6 days (see Table 4.2), while SOS for Shannon was 15.6 days. Likewise, SFG varied among sites (Table 4.2) and did not always correspond to contaminant-based status. Wexford had the lowest LT_{50} indicating potential stress at this site however none of the initially selected contaminants exceeded the EAC. Condition factor also varied and also did not align with the contaminant results. Tralee Bay had the lowest condition factor but this may have been linked to a high infestation of pea crabs.

Table 4.2 Scope for growth (J g^{-1} h^{-1}) and SOS response (LT_{50}) data for the Tier 1 sites with applied assessment criteria. Colour key < BAC-Blue, >BAC<EAC/EC-red as per relevant assessment criteria.

Location	SFG	SOS
Wexford Harbour	4.75	8.79
Bantry Bay	7.75	10.3
Dublin Bay	4.38	13.3
Cork Harbour		17.6
Tralee Bay		9.89
Kinvarra Bay		12.5
Shannon Estuary	10.01	15.6
Galway Bay	13.6	11.4
Newquay	3.62	9.59
BAC	15	10
EAC	5	5

The method for assessment of SFG data is outlined in Chapter 3 and uses the mean SFG + the 95% confidence interval. The difference in assessment with using the mean or median + 95% confidence interval could potentially pose problems when assigning status around the good, not good boundary (blue or green to red). There may be uncertainty in assigning status around the not good:good boundary in relation to whether the SFG is reduced with contaminant burden. This raises potential difficulties not only in the biomarker/contaminant load relationship but also in the reliability of the biomarkers themselves and hence barriers meeting compliance for MSFD.

Principal component analysis of all nine sites with SOS, CR and CI revealed that Cork and Wexford separated furthest from each other (Figure 4.2). Also, PCA analysis with six sites data with SFG and RR showed Wexford, Dublin and Newquay separated furthest from Galway (Figure 4.3).

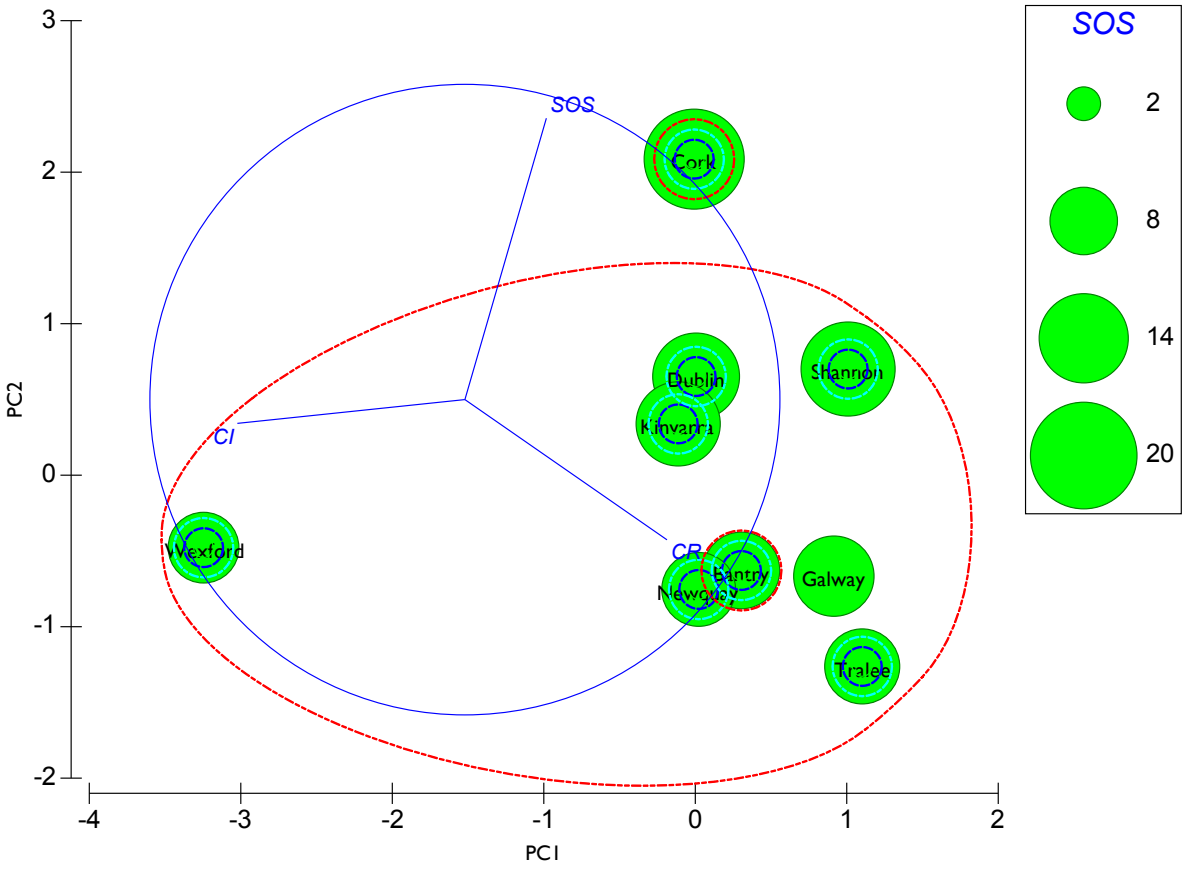


Figure 4.2 Principal components analysis of biological effects in *Mytilus edulis* sampled from nine Tier 1 locations including SOS, CR and the supporting parameter CI with SOS as main key. Red line indicates % similarity.

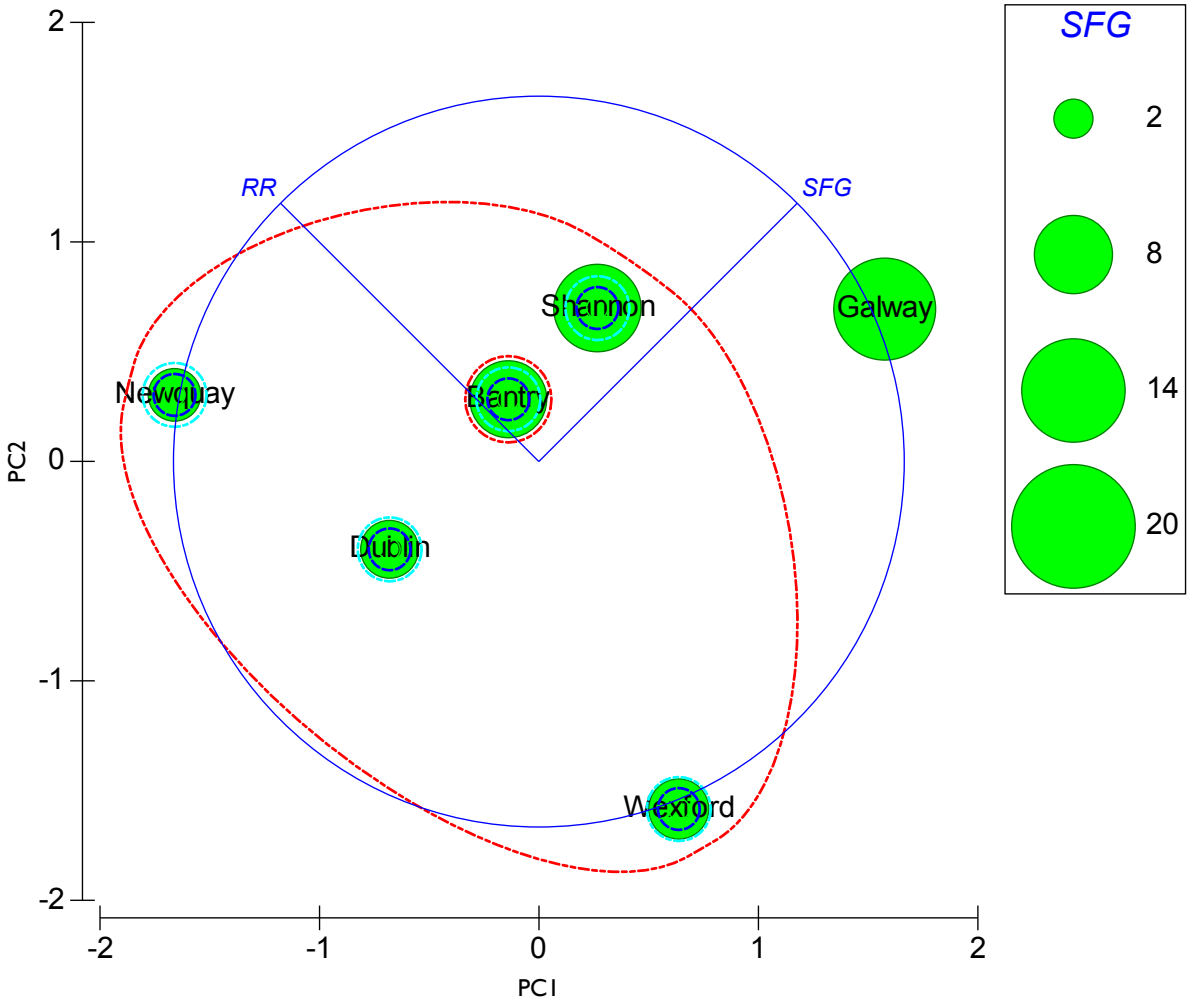


Figure 4.3 Principal component analysis of biological effects in *Mytilus edulis* sampled from six Tier 1 locations including SFG and RR with SFG as main key

4.2.1.2 Sediment Chemistry and Ecotoxicity Testing of Tier 1 Sites

The sediment chemistry analysis showed Dublin (Tolka estuary) and Cork (Ringaskiddy) were the most contaminated locations especially with respect to PCBs and metals. Bantry exhibited EAC exceedances for lead, copper and zinc and it should be noted that elevated p'p'-DDE was detected in sediment in Bantry. Both Kinvarra and Tralee sediment showed elevated levels of copper. In general, most sites had levels of contaminants below the EAC.

Table 4.3: CEMP contaminants (µg kg^{-1} dry weight) in sieved sediment (<63µm) at Tier I locations around the Irish coast. Colour key: <BAC-blue, >BAC<EAC/EC-green, >EAC/EC red, as per relevant assessment criteria.

	Shannon (TI/II)	New Quay	Tolka	Tralee	Cork	Kinvarra	Bantry	Wexford (TI/II)	Cork (TII)	Dublin Bay (TII)
PCB 28	0.06	0.06	2.3	0.23	20.2	0.06	0.41	0.23	0.18	1.39
PCB 52	0.03	0.03	5.3	0.04	8.52	0.04	0.49	0.03	0.03	0.33
PCB 101	0.04	0.03	5.4	1.21	4.61	0.05	0.65	0.04	0.04	0.14
PCB105	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
PCB 118	0.02	0.02	6.2	2.78	4.05	0.04	0.97	0.02	0.04	0.23
PCB 138	0.09	0.09	6	2.89	2.53	0.09	1.41	0.66	0.5	0.39
PCB 153	0.09	0.09	4.1	1.31	1.96	0.09	3.11	0.53	0.41	0.37
PCB156	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.03	NA	0.03
PCB 180	0.06	0.02	1.3	0.29	0.31	0.04	NA	0.03	0.33	0.26
gamma-HCH	0.04	0.04	0.14	0.03	0.04	0.04	0.02	0.03	0.04	0.04
alpha-HCH	0.12	0.05	0.11	0.05	0.31	0.07	0.06	0.71	0.56	0.18
4.4´-DDE	0.03	0.02	0.54	0.013	0.02	0.04	3.41	0.01	0.03	0.26
HCB	0.05	0.05	3.3	0.049	0.03	0.07	0.12	0.13	0.04	0.04
Dieldrin	0.04	0.01	0.8	0.013	0.01	0.04	0.73	0.01	0.01	0.13
Naphthalene	NA	7.27	81.6	5	NA	NA	NA	NA	69.9	119
Phenanthrene	NA	8.68	147	43.9	NA	NA	NA	NA	38.2	225
Anthracene	NA	1.27	58.8	1.36	NA	NA	NA	NA	1.8	53
DBT	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Fluoranthene	NA	9.8	244	38.1	NA	NA	NA	NA	37.1	283
Pyrene	NA	7.79	268	35.8	NA	NA	NA	NA	59.7	338
Benzo(a) anthracene	NA	3.39	124	1.94	NA	NA	NA	NA	3.7	144
Chrysene	NA	3.86	146	2.56	NA	NA	NA	NA	16.6	169
Benzo(a)pyrene	NA	3.57	172	1.41	NA	NA	NA	NA	4.7	192
Benzo(ghi) perylene	NA	2.69	136	1.13	NA	NA	NA	NA	4	126
Indeno(1,2,3-c,d)pyrene	NA	3.93	148	1.5	NA	NA	NA	NA	14.6	138
Cd	220	470	1060	490	390	930	550	40	60	140
Hg	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Pb	6930	7990	43600	21500	91600	24600	142000	4450	16600	10400
As	11300	15500	15800	18000	8590	18000	14900	5300	32900	5290
Cr	18400	21700	72100	21200	20300	30900	27300	8980	19600	18000
Cu	2600	4990	72300	53100	10700	94700	71900	1770	4440	9910
Ni	11300	11600	43100	29100	17000	17300	30300	5460	10200	11100
Zn	18600	18700	172000	69400	92000	61500	223000	14200	33000	38100

NA:Not analysed, TII = Tier II

Very few LC_{50s} could be calculated from the Tier 1 sediment elutriate samples with the amphipod *Tisbe battagliai* (TB), the diatom *Skeletonema costatum* (SC) or bacteria *Vibrio fischeri* (VF) due to low effect levels. Porewaters on the other hand did elicit some effects with SC being the most sensitive and showing 100% inhibition of growth at Galway, Newquay, Dublin Bay, Kinvarra and Wexford. It should be noted that competition with opportunistic algal spp. may have affected these porewater results hindering interpretation. There was a 39% inhibitory effect on VF by porewater from Galway sediment. With the whole sediment tests, Wexford sediment was the most toxic eliciting a 73% mortality and 87% inhibition of production of faecal casts in *Arenicola marina*. There were no LC_{50}s determined with *Corophium* and any of the sediment samples and the highest percentage mortality was determined with sediment from Tralee (LC_{50}: 28%). Principal components of the whole sediment test data is shown in Figure 4.4 below.

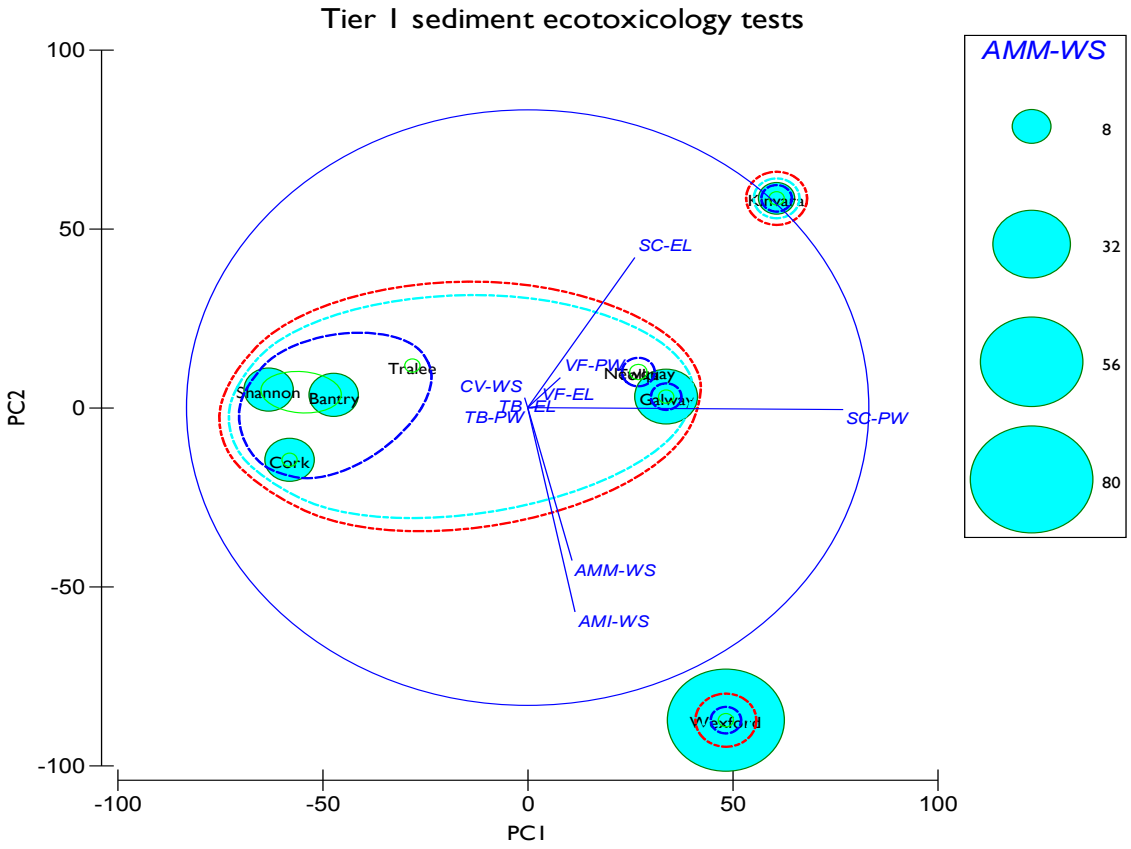


Figure 4.4: Principal components analysis showing data projections for sediment ecotoxicology tests including TB, SC and VB tests with –EL and -PW. Also *Corophium volutator* (CV) and *Arenicola marina* (AM) mortality (-M) and inhibition (I) with whole sediment (-WS) with principal legend for Arenicola marina (AM) mortality in whole sediment AMM-WS for the nine Tier 1 locations.

A range of responses were detected at various sites with both *A. marina* and *C. volutator* whole sediment tests. It is clear from the PCA that Wexford separated furthest from the other sites and showed the highest percentage mortality with *A. marina*. Kinvarra separated in relation to high

responses in *S. costatum* growth inhibition after exposure to both porewater and elutriates. There was no clear picture for the other sites.

4.1.3. Overall Outcomes of Tier I Sampling and Analysis

Overall the contaminant and biological effects rankings did not correlate/complement each other with regard to ranking of sites with a) contaminants and b) biological effects. Based on these data in addition to available historical data from these sites, Wexford, Dublin and Cork and the control at Shannon were chosen for application of the wider test battery approach in line with the proposed Tier II approach.

4.1.4. Tier II Results

4.1.4.1 Biomarkers in the Mussel *Mytilus edulis* at Four Tier II Locations

The biomarkers MT, AChE and Alkali labile phosphate were used to assess Tier II mussels and were sampled at the same time as Tier I mussels in order to assess differences between organizational levels within the individual. Metallothionein data is not presented for the purposes of this report due to high variability in the results (Rochford, 2012). Alkali labile phosphate results are further discussed in Case study 2 on EDCs in Ireland. AChE measurements in *M. edulis* from all sites were below EAC with Wexford and Cork found to exceed the BAC.

4.1.4.2 Biomarkers in Dab (*Limanda limanda*) and Plaice (*Pleuronectes platessa*) at Four Tier II Locations

All dab sampled from Shannon, Cork Harbour, Wexford and Dublin all showed EROD activities significantly below the background criteria utilised and levels were comparable to levels determined in flounder in UK waters (Kirby et al. 1999). Male plaice samples from Dublin Bay (DB-3) were determined to be above the BAC at this location (see Figure 4.5). There were significant differences between male and female fish at Wexford.

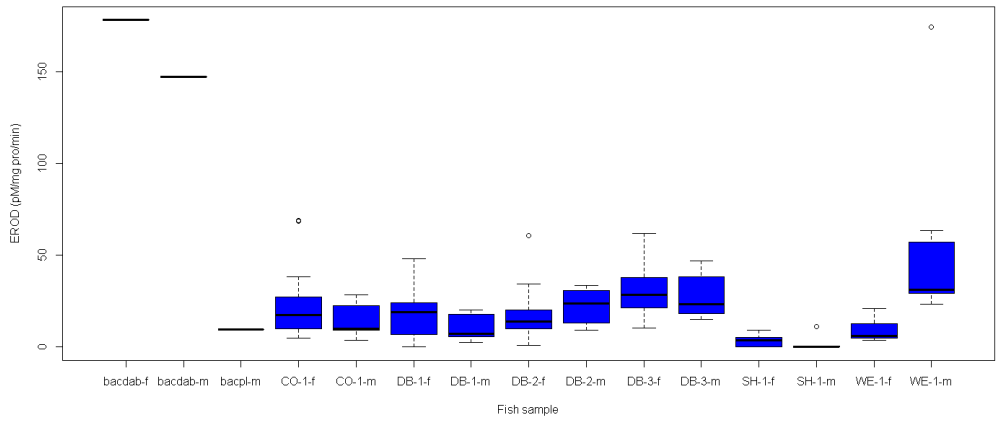


Figure 4.5 O-deetylase 7-Ethoxyresorufin (EROD) in fish samples from four Tier II locations around the Irish coast. Sampling locations are as per Table 4.3.

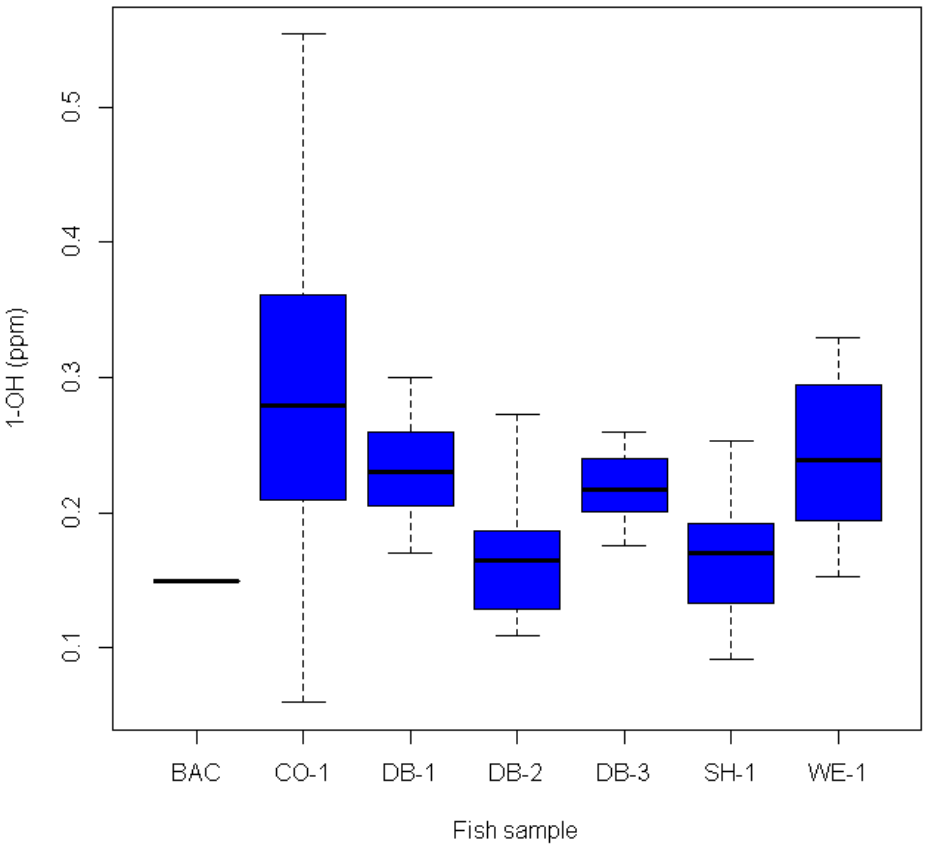


Figure 4.6 Bile metabolites in fish sampled from four Tier II locations around the Irish coast. EAC not shown on graph (EAC: 22ppm). Sampling locations are as per Table 4.3.

Bile metabolites in dab samples, reported as 1-hydroxypyrene equivalents in this study were all determined to be significantly below the EAC but above BAC at the four sites monitored.

Low acetylcholinesterase (AChE) enzyme activity is indicative of a higher level of pollution. Acetylcholinesteras activity was measured in dab samples (see Figure 4.7) with lowest activity observed in Shannon (and above EAC) and higher activity (lower than BAC) observed in Wexford and Dublin, with Cork levels above BAC but below EAC. These results were in contradiction to the chemical analysis results which showed all pesticides to be below EAC with the exception of p,p'-DDT. The low level EAC for p,p'- DDE was exceeded at Dublin, Cork and Wexford however this is reflective of the overall profile in most OSPAR regions. Organophosphate and carbamate insecticides are potent neurotoxins and known to block the breakdown of acetylcholine (ACh) by the enzyme, acetylcholinesterase however no definitive relationship was detected between OCs measured.

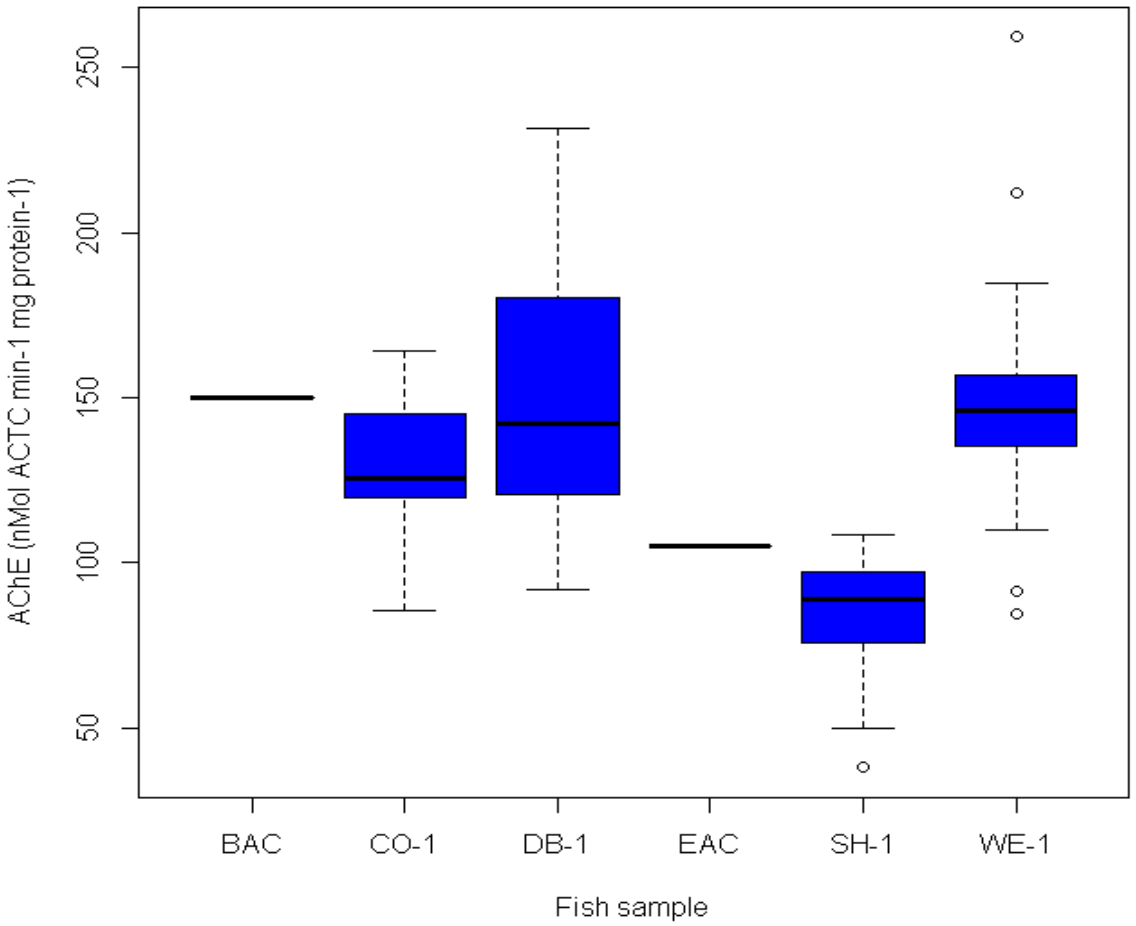


Figure 4.7 Acetylcholinesterase activity (nMol ACTC min^{-1} mg $protein^{-1}$) in fish muscle samples with associated BAC and EAC in dab sampled from four Tier II locations. Sampling locations are as per Table 4.3. Note: Outlier (874.94 nMol ACTC min^{-1} mg $protein^{-1}$) at Wexford was removed for graphical visualisation purposes

4.1.4.3 Fish Histopathology

Prevalence of pathologies (histopathological liver lesions in fish) in livers of dab and plaice sampled from the four Tier II locations are shown in Table 4.3. Overall, fish populations of dab from Cork Harbour, Dublin Bay and Shannon had the highest prevalence of liver lesions associated with the carcinogenic pathway Foci of Cellular Alteration (FCA), Benign Neoplasms (BN) and Malignant Neoplasms (MN). Dublin Bay showed the highest levels of FCA. It should be noted that numbers of fish were low in Cork Harbour and therefore there is lower confidence associated with these results. Analysis completed on a sample number of 56 individuals is sufficient to indicate a 95% confidence for the prevalence of each of these liver lesion categories. Insufficient numbers of individual fish were available at each location (recommended n>250) in order to detect external diseases in fish samples. Any external diseases or parasites were noted onboard during sampling. Wexford had the most diverse quantity of external diseases including glugea, ulcerations, fin rot, papillomas and scoliosis. Ulcerations were also noted at the Dublin Bay and Cork sites. Cork and

Wexford contained the highest levels of PAHs in bile. Male dab in Wexford showed the most elevated EROD response. Further investigation is warranted into the investigation of fish disease and biomarker studies in future monitoring.

Table 4.4 Prevalence % of pathologies recorded in the livers of dab and plaice (autumn 2010) at the four Tier 1 locations including non specific lesions (NSL), early toxicopathic non-neoplastic lesions (NNT), Foci of Cellular Alteration (FCA), Benign Neoplasms (BN) and Malignant Neoplasms (MN).

Location	Code	Species	n	NAD	NSI	NNT	FCA	BN	MN
Cork Harbour	CO-1	Dab	23	82.6	8.7	0.0	4.3	4.3	0.0
Dublin Bay	DB-1	Dab	23	26.1	30.4	0.0	34.8	4.3	0.0
Dublin Bay	DB-2	Dab	50	32.0	22.0	0.0	34.0	6.0	4.0
Dublin Bay	DB-3	Plaice	20	40.0	35.0	0.0	25.0	0.0	0.0
Shannon	SH-1	Dab	56	41.1	41.1	1.8	8.9	5.4	5.4
Wexford	WE-1	Dab	47	42.6	36.2	0.0	0.0	0.0	0.0

4.1.4.4 Sediment Ecotoxicology

Sediments from the fish sampling locations were investigated for toxicity. Results of the four locations are reported in Table 4.5. Effects (above LC_{50}) were elicited in *Skeletonema costatum* (SC) with porewater and elutriate however these effects could be due to competition with opportunistic species present. With the exception of SC testing no samples were found to be above their relevant EAC. Cork and Shannon sediment had effects above BAC but below EAC for *Arenicola marina* testing.

Table 4.5: Effects (% inhibition and % mortality) for porewater, elutriate and whole sediment tests on *Tisbe battagliai (TB), Skeletonema costatum (SC), Vibrio fischeri (VF), Corophium volutator* (CV) and *Arenicola marina (AM)* on four Tier II locations.

	Elutriate			Pore-Water			Whole Sediment		
Site	TB	SC	VF	TB	SC	VF	CV	AMM	AMI
Wexford	0	2	0	0	100	0	0	6.7	0
Cork	0	83	0	0	100	0	3.3	40	33
Shannon	0	0	0	0	0	0	0	46.7	23
Dublin	0	0	0	0	100	17	26	7.6	0

4.1.4.5 Imposex in Marine Gastropods

An imposex survey was undertaken in 2011 incorporating a range of sites in addition to the Tier II sites. This was additional work and was funded by the Marine Institute and EPA. Details of this study can be found in Case Study One.

4.1.5. Overall Outcomes

Overall very low levels of biological effects were encountered at Tier II locations in dab, plaice, and mussels and in sediment dwelling organisms. For sediment toxicity, there is high variability in sediment particle size and lack of background data makes it quite difficult to derive accurate assessment criteria for future assessments. For integration purposes, it is important that samples are taken within close proximity to each other. It should be noted that fish and sediment samples for Tier II were taken at quite a distance from the mussel beds. For future monitoring it is recommended that species residing nearer to the shoreline be investigated (e.g. flounder which reside in estuaries for longer periods than dab). It is clear from the fish histopathology results that further investigations are warranted for the future in order to elucidate cause and effect relationships or whether other factors contributed to the observed effects. Further conclusions and recommendations with respect to individual aspects are discussed in chapter seven.

5. Primary case studies: Chapter five

5.1. Case Study One: Imposex in the Dogwhelk *Nucella lapillus* around the Irish Coast

5.1.1. Background

Tributyltin (TBT) has historically been used as a paint additive to prevent biofouling growth of aquatic organisms on for example, ship hulls. It is one of the most toxic contaminants found in the marine environment. TBT leaches from the paint resulting in pollution of harbours, ports and coastal areas (Fent, 1989). It is toxic to many marine organisms and has been linked to reductions in meat weight and shell distortion in oysters (Alzieu et al., 1982). It has been shown to have effects such as limb deformities in crab species, arm regeneration in brittle stars and induction of high larval mortality of the common mussel (Forsyth and Casey, 2003). In Ireland, grossly distorted oysters were reported from Cork Harbour and Baltimore, Co. Cork in 1985 (Minchin et al., 1987) and year-class failures of 1992-1995 of scallops in Mulroy Bay. Concern was raised that TBT released from salmon cages used in local aquaculture treated with a TBT flexible net-dip coating could have adverse consequences for local aquaculture and scallop ranching (Minchin, 1995). As a result, an Irish Bye-law was passed in April 1987, prohibiting the use of TBT on all vessels under 25 m except under special circumstances. There has since been a complete prohibition on TBT use since 1st January 2008 which has been implemented in the EU by council Directive 2002/62/EC. The most notorious effect of TBT is the imposition of male characteristics on female gastropods, known as imposex. This has now been adopted as a more sensitive, quantitative and reproducible biomarker for TBT contamination, under the OSPAR JAMP Guidelines (OSPAR 2008) and is one of the key components of the integrated approach (ICES, 2012). Table 5.1 below shows interpretations of assessment classes of imposex using the vas deferens sequence index (VDSI) in *Nucella lapillus* as an indicator. OSPAR assessment criteria indicate VDSI < 0.3 (A) as near background and VDSI > 2 (C-F) as exceedence of EAC.

This investigation, which was carried out in 2011, is part of the monitoring process initiated by the OSPAR program in 1987 to monitor TBT contamination in order to establish the current status around the Irish coast and the temporal trends at selected locations. An assessment of the status and trends up to 2009 (McGovern et al. 2011) showed that, while the great majority of the sites failed the Vas Deferens Sequence Index VDSI ecological quality objective EcoQO status (i.e. VDSI > 2.0), over half of the sites showed some signs of improvement. This case study aimed to investigate effects of the ban of TBT on populations of dogwhelks.

Table 5.1 Interpretations of the assessment classes, which refer to *Nucella lapillus* representing the most TBT sensitive gastropod species used in the OSPAR JAMP monitoring guidelines (OSPAR 2008)

Class	Nucella VDSI	Effects and impacts
A **BAC**	<0.3	The level of imposex in the more sensitive gastropod species is close to zero (0 - ~30% of females have imposex) indicating exposure to TBT concentrations close to zero, which is the objective in the OSPAR hazardous substances Strategy.
B	0.3 - <2.0	The level of imposex in the more sensitive gastropod species (~30 - ~100 % of the females have imposex) indicates exposure to TBT concentrations below the EAC derived for TBT. e.g. Adverse effects in the more sensitive taxa of the ecosystem caused by long-term exposure to TBT are predicted to be unlikely to occur.
C **EAC**	2.0 - <4.0	The level of imposex in the more sensitive gastropod species indicates exposure to TBT concentrations higher than the EAC derived for TBT. e.g. There is a risk of adverse effects, such as reduced growth and recruitment, in the more sensitive taxa of the ecosystem caused by long-term exposure to TBT.
D	4.0 - 5.0	The reproductive capacity in the populations of the more sensitive gastropod species, such as *Nucella lapillus*, is affected as a result of the presence of sterile females, but some reproductively capable females remain. e.g. There is evidence of adverse effects, which can be directly associated with the exposure to TBT.
E	> 5.0	Populations of the more sensitive gastropod species, *Nucella lapillus*, unable to reproduce. The majority, if not all females within the population have been sterilised.
F	VDSI = -	The populations of the more sensitive gastropod species, such as *Nucella lapillus* and *Ocinebrina aciculata*, are absent/expired.

Figure 5.1: Imposex sampling locations (left) NBL Dublin (right) and male *Nucella lapillus* removed from its shell (centre)

5.1.2. Materials and Methods

From autumn 2010 – autumn 2011, *N. lapillus* were collected from 63 stations around Ireland (Fig. 5.1). Relative penis size index (RPSI) and VDSI were determined using international standard techniques (OSPAR, 2002). Temporal assessment was performed on 10 areas [Carlingford, Dublin Bay, Wexford, Waterford, Cork, Castletownbere, Tralee Bay, Killybegs, Ballinakill Bay and Mulroy Bay] with data from a number of surveys between 1987 and 2011 (Minchin and Minchin 1997; Minchin et al. 1995; Minchin et al. 1996; Minchin et al. 1997; Minchin, 2003; Minchin, 2011; Giltrap et al. 2009). Also, changes in individual areas [Dublin Bay, Killybegs and Cork] were investigated. A mixed effects model was used to determine whether VDSI values changed for all areas between 1987 and 2011. Sampling dates were divided into three categories: early (1987-1995), middle (1996-2004), and recent (2005-2011).

5.1.3. Overall Findings and Conclusions

The majority of the sites from each of the 10 locations surveyed met the EcoQO, that is had a VDSI of <2.0. However, it is noted that only in Galway Bay, Shannon, Ballinakill Bay and Tralee Bay did all individual sites meet the EcoQO. High levels of imposex (VDSI stages 5 and 6) indicative of sterility in females, were found at Castletownbere (all sites), Waterford (3/4 sites), Cork harbour (5/8 sites), Tralee Bay (1/4 sites) and Killybegs (1/7) sites. Dogwhelk (Nucella lapillus) populations had not recovered at Walkers Bay in Killybegs which had been previously surveyed in 1994/95 where the absence of dogwhelks was noted. Also, no dogwhelks had recovered where they were at one time present at Dooneen Pier in Ballinakill Bay in Co. Galway. The extinctions at Walkers Bay and Dooneen Pier are due to local geographical exclusion and not due to continuing contamination. With one or two exceptions, there has been a dramatic improvement in the reduction of organotin contamination status around the whole of the Irish coast since the partial ban in 1987 and complete ban in 2008 suggesting measures implemented nationally and internationally have been effective in reducing TBT pollution of Irish coastal waters. This improvement is relatively rapid and suggests that those places which are still showing signs of contamination (i.e. high VDSI scores) either are subject to different hydrological regimes, such that organotins are not removed but continue to accumulate in the sediments, that organotin inputs are persisting or that there are other contaminants which can induce endocrine disruption. For the MSFD, imposex in dogwhelks is one of the key biological effects tools for investigation of good environmental status in Descriptor 8. Despite the significant downward trend in impact from TBT, areas in the 2010/2011 study which showed non compliance with the EcoQO should be subject to future monitoring. It should be noted that international research is ongoing in relation to dumpton syndrome. While outside of the scope of this project this genetic mutation appears to protect the female from imposex and has been reported to have started to reverse population declines and should be taken account of in future studies. This case study will be the subject of a more extensive peer review and is presently in draft form for publication.

5.2. Case Study Two: A Summary Assessment of the Presence and Effects of EDCs in Irish Marine Waters

5.2.1. Background

A wide range of natural and synthetic contaminants has been shown to affect reproduction, growth, development and immune system response in exposed organisms. Such contaminants have been termed endocrine disrupting compounds (EDCs). EDCs may be released into the aquatic environment via a number of sources, including municipal and industrial waste water treatment plant effluent (WWTPE), agricultural and terrestrial run off, and/or accidental release. Few data documenting EDCs loadings and associated effects are available for the Irish marine environment. This study developed analytical techniques capable of measuring trace levels of selected EDCs in marine waters and biota and further completed a limited chemical and biological effect based assessment.

The naturally occurring steroid estrogens estrone (E1) and 17β estradiol (E2), the synthetic estrogen used in the contraceptive pill 17α ethynylestradiol (EE2) and synthetic compounds nonylphenol (NP) and octylphenol (OP) which are used in the manufacture of antioxidants, as lubricating oil additives, and in the production of alkylphenol ethoxylates, were selected as target compounds for analysis due to their documented effects in the laboratory and in the environment, and on their legislative relevance. There is a requirement to monitor NP and OP under the WFD 2000/60/EC while both E2 and EE2 are currently proposed for inclusion in the WFD list of priority pollutants.

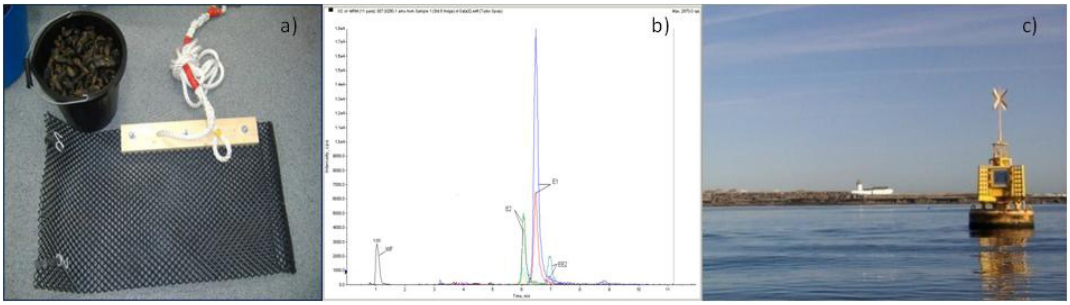


Figure 5.2: a) Caging technique used to deploy shellfish, b) typical LC-MS/MS chromatogram depicting EDCs, c) Mutton Island Galway test site.

5.2.2. Materials and Methods

Shellfish transplantation techniques were developed to test the feasibility of completing deployments in areas where biota may be absent. This was further complimented by the analysis of resident shellfish, native fish (dab and plaice), water and sediment from a range of pilot sites in both impacted and lesser impacted Irish coastal locations. Within the confines of available assessment criteria (see Appendix 2) a 'weight of evidence' approach including 'traditional' chemical analysis methods, biological effects monitoring and more recently developed passive sampling technologies was

then employed to make a summary "integrated" assessment of the presence of EDCs and level of endocrine disruption (ED) at test sites.

5.2.3. Overall Findings and Conclusions

EDC concentrations were measured according to the methodology of Ronan and McHugh (2013) and Ronan (2013). Results indicate that the presence and effects of EDCs at the selected sites were generally low. In the case of Vtg one male individual had high Vtg in Dublin Bay and additionally a number of individual male fish from Wexford were also found to exhibit higher Vtg levels. Low levels of steroids were detected in Dublin Bay water samples, and ALP levels were highest in Wexford. Significant differences in ALP (an indirect measure of response to estrogenic EDCs) were noted between sites however no direct link between proximity to potential sources of EDCs and ALP levels were found. E2 water levels at three sites studied were below proposed WFD annual average environmental quality standard (AA EQS) total water values, thus the sites could be considered to have low ecological risk status in terms of E2. POCIS passive samplers were utilised as a valuable screening tool and derived pollutant profiles supported the analytical chemistry in terms of similar pollutant profiles.

Future EDC related analysis should consider continual lowering of analytical detection limits in order to keep with low proposed EQS values in water and the potential for future EDC EQS in biota. As quantitative analysis of polar compounds by PS improves there is an obvious role for the technique in the provision of time integrated analytical data.

Alkali labile phosphate and ER-LUC were found to be valuable tools for assessing ED effects in the environment, accounting for the effects of many compounds not selected for by targeted chemical analysis. At an organism level further work is merited to elucidate EDC related modes of action in marine biota and on the potential influence of natural estrogen levels in test species. Such information would enhance the application of biomarkers in the field of pollutant monitoring and enable accurate risk assessment and must also be incorporated into any proposed monitoring programmes.

Overall levels and effects reported in this limited pilot study are generally low. Combined chemical and biological effects measurements suggest that further monitoring of steroid and EDC levels in water and ED effects in resident species may be merited in areas such as Dublin Bay and Wexford. Chemical and biological analyses were found to be complimentary tools for the investigation of EDCs and ED in the marine environment. The combination of techniques used provided a cost effective means of assessment, with a range of analyses applied to a single sample. While the listed suite of EDCs and associated effects techniques are generally not being considered for inclusion into MSFD assessment approaches their relevance with respect to WFD monitoring and for ongoing inclusion in future integrated monitoring programs (especially those subject to potential point source exposure) should be considered.

Table 5.2: Concentrations of EDCs in marine matrices and a pilot assessment, POCIS = ng device^{-1}, water ng L^{-1}, tissue ng g^{-1} wet weight, ALP in µg mg protein^{-1}, Vtg µg ml^{-1} plasma in male fish.

			POCIS			Water			Tissue							
Location	Matrix	Matrix	E1	E2	EE2	E1	E2	EE2	E1	E2	EE2	NP	OP	ng EEQ	ALP	Vtg
Cork	Shellfish(n=2)	Mussels							nd	nd	nd	nd	nd	0.08	12.5	
	POCIS (n=2)	POCIS	4.12–5.18	0.12–0.49	<0.40											
	Fish (n=6)*	Dab							nd	nd	nd	nd	nd			0.2
	Sediment	Sediment							*	*	*	*	*	0.1		
Dublin Bay	Shellfish (n=12)	Mussels							nd	nd	nd	nd	nd			
	Shellfish	Mussels							nd	nd	nd	nd	nd	0.24 - 0.72	7.5 - 14.81*	
	POCIS (n=4)	POCIS	3.59-8.45	0.69-3.8	<0.10											
	Water (n=5)	Water				0.76-1.11	nd-0.13	nd								
	Sediment	Sediment												0.29		
	Fish (n=4)	Dab + plaice							nd	nd	nd	nd	nd			0.2-373**
Shannon	Shellfish (n=1)	Mussels							nd	nd	nd	nd	nd	0.38	12.8	
	POCIS (n=1)	POCIS	<1.0	<0.30	<0.10											
	Fish (n=3)*	Dab							nd	nd	nd	nd	nd			0.2
Wexford	Shellfish (n=2)	Mussels							nd	nd	nd	nd	nd	0.102	40.22	
	POCIS	POCIS	6.5	1.61	<0.40											
	Fish (n=2)*	Dab							nd	nd	nd	nd	nd			0.2-0.44
	Sediment	Sediment												0.21		

1 n=2 samples 2 n=1 sample, * Fish species tested dab and plaice. Chemical analysis conducted on muscle and liver tissue. LOD fish muscle for E1, E2, EE2: 0.4, 0.9, 0.3 ng g^{-1}, respectively. LOD fish liver 0.02, 0.09, 0.2µg g^{-1} for E1, E2, EE2, respectively. LOD shellfish: 0.4 0.9 0.3 ng g^{-1}, for E1, E2, EE2 respectively. All LODs wet weight. LOD water 0.07, 0.07, 0.11 ng L^{-1} for E1, E2, EE2 respectively. ** 3 of 28 male fish samples had elevated Vtg at 109, 167 and 373 µg ml^{-1} Vtg. 9 fish between 0.3 and 4.8 with the rest at 0.2 µg ml^{-1}. The remainder (n=16) at 0.2 µg ml^{-1}.

5.3. Case Study Three: Application of Passive Sampling Methodologies to Support Environmental Monitoring

5.3.1. Background

Passive sampling (PS) methodologies are fast being recognised as having the potential for sensitive, time-integrated monitoring of micropollutants in the aqueous phase thereby acting as viable alternatives to conventional sampling techniques. PS measurements while not directly comparable in many cases to quality objectives or assessment criteria (e.g. as derived for the WFD) are relevant in environmental quality assessment (e.g. OSPAR). The working group for biological effects of contaminants (WGBEC) (ICES, 2007) acknowledged the advantages of combining the use of PS and "bioanalyses" as important links between WFD and MSFD and recommends their use.

Passive sampling as reported in this project employed POCIS devices for the identification of steroidal compounds and also PDMS strips for the determination of dissolved concentrations of hydrophobic pollutants. Results of screening using POCIS are reported elsewhere in this report. This project sought to further develop the capacity to complete PS based analysis for a number of priority pollutants, to deliver trace level "baseline" contaminant levels in Irish coastal waters and to then where feasible incorporate analytical data into the wider "integrated" assessment.

The use of PDMS has now becoming a commonly used technique for monitoring a range of hydrophobic pollutants in marine environments. PDMS was successfully deployed at all Tier II sites (Dublin, Cork,, Wexford and Shannon) and further complimented by additional deployments at Bantry and Galway (Mutton Island) with a "reference" deployment completed at Omey Island on the west coast, with data from this location providing a context in terms of low anthropogenic inputs. Analysis and modelling was completed in accordance with current best practice as per Smedes et al (2009) and using ICES guidelines developed by Smedes and Booij (2012). The dissolved water concentration for a number of persistent pollutants at each of the sampling locations are presented below.

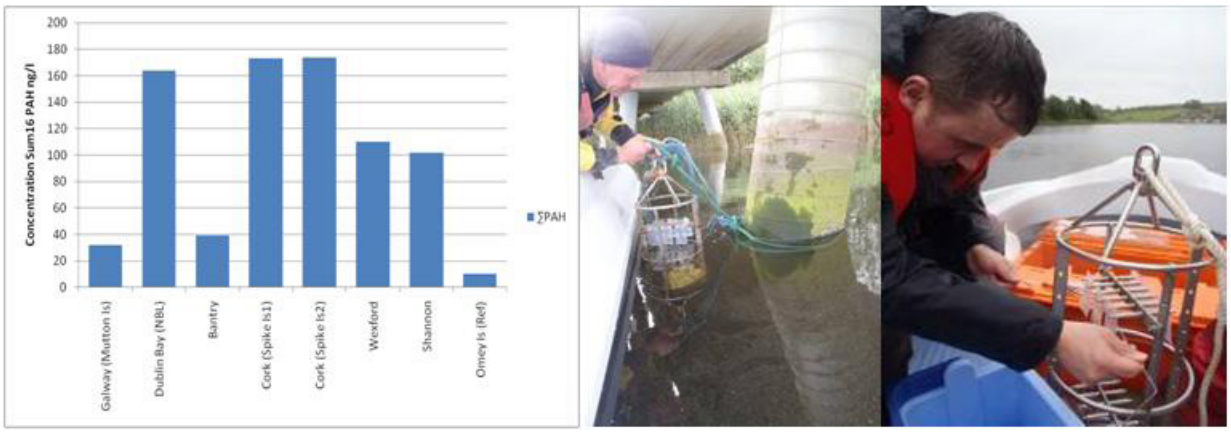


Figure 5.3: Dissolved concentrations (ng l^{-1}) of Σ16US EPA PAHs at Irish marine locations. Preparation and deployment of PDMS samplers.

Table 5.3 Passive sampling derived concentrations for indicative persistent pollutants (ng l^{-1}).

	Galway (Mutton Island),	Dublin Bay (NBL)	Bantry	Cork (1)	Cork (2)	Wexford	Shannon	Omey Island
Acenaphthylene	0.77	3.24	1.36	1.11	0.85	1.70	1.61	0.30
Acenaphthene	0.33	2.64	0.57	2.02	1.37	4.32	1.13	0.14
Fluorene	0.91	4.12	2.85	5.32	3.90	3.12	4.50	0.67
Phenanthrene	2.08	4.85	2.88	9.48	8.67	4.67	12.44	1.27
Anthracene	0.16	0.89	0.16	0.48	0.63	1.26	0.70	0.05
Fluoranthene	1.02	5.47	0.66	5.60	4.98	5.45	5.97	0.52
Pyrene	0.56	6.10	0.26	3.39	3.07	3.32	3.43	0.10
Chrysene	0.06	1.09	0.01	0.47	0.41	0.75	0.19	0.02
Benzo(a)anthracene	0.12	1.64	0.05	1.17	1.06	1.43	0.57	0.04
Benzo(b) fluoranthene	0.04	0.89	0.02	0.76	0.67	0.88	0.11	0.02
Benzo(k) fluoranthene	0.03	0.54	0.02	0.76	0.64	0.77	0.11	0.02
Benzo(a)pyrene	0.02	0.67	0.00	0.28	0.22	0.19	0.04	0.01
Indeno(1,2,3-cd) pyrene	0.01	0.19	0.01	0.47	0.31	0.18	0.03	0.01
Dibenzo(a,h) anthracene	0.01	0.07	<0.01	0.11	0.05	0.04	<0.01	<0.01
Benzo(g,h,i)perylene	0.01	0.24	<0.01	0.39	0.27	0.14	0.03	<0.01
PCB 153*	0.05	0.26	0.02	0.14	0.15	0.03	0.04	0.02
HCB**	0.09	0.11	<0.01	0.08	0.08	0.10	0.13	<0.01
PPDDE	0.02	0.04	<0.01	0.08	0.09	0.06	0.01	**0.10**

*PCB 153 indicative of total PCB loading. **AA-EQS for HCB 10ngl^{-1}.Cork (1 and 2) (Spike Island replicate samples) respectively.

Overall the Σ15PAH was more elevated in more industrial areas relative to the Omey Is. reference site. Heavier PAHs such as phenanthrene, fluoranthene and pyrene predominate in areas with greater marine traffic and general industrial influences. The observed profile in PS devices broadly reflects that for sentinel mussels for the same locations. Hexachlorobenzene (HCB and PCB loadings were found to be low with HCB being below the respective AA-EQS for the parameters.

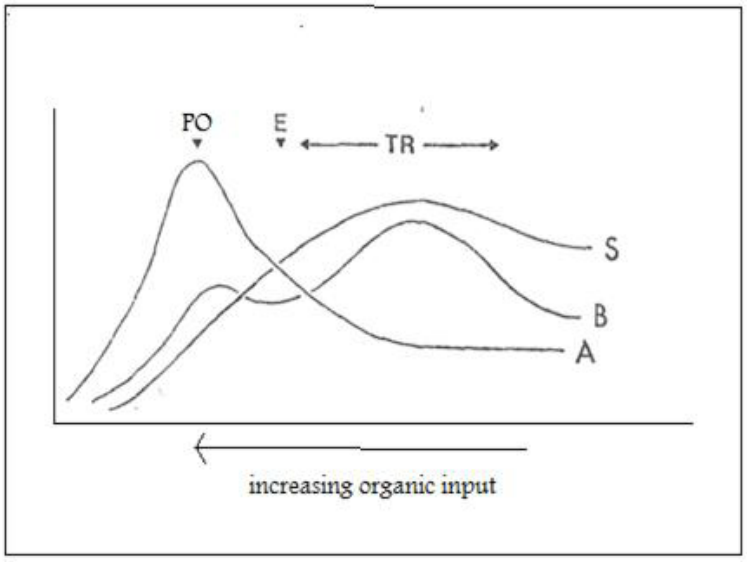
Passive Sampling based techniques allow for the determination ultra trace "time-integrated" contaminant levels in marine waters this being an impractical task using conventional spot water sampling. The techniques measure dissolved water concentrations thus comparison to WFD derived EQS are not directly possible, it is however generally understood that the dissolved fraction is the most relevant in respect of supporting ecotoxicological assessments. The relevance of the emerging area of passive dosing whereby PS devices are exposed *in-situ* and are then extracted to provide test samples for bioassay based analytical techniques looks likely to increase in future years. While agreed PS derived assessment criteria are currently still unavailable, the use of PS techniques to support

integrated assessments is strongly supported within ICES and OSPAR. It is recommended therefore that passive sampling continues to form a core part of future Irish integrated monitoring programs in support of WFD and MSFD monitoring commitments.

5.4. Case Study Four: Benthic Indices as a Tool for Pollution Monitoring

5.4.1. Background

A major advantage of using macrobenthic communities as indicators of pollution is their spatial and temporal stability (Pearson and Rosenberg, 1978). Their conceptual model (Figure 5.4) showing how species number (S), total abundance (A) and total biomass (B) change along a gradient of increasing organic input has provided much of the basis for current marine benthic indices.



On the heavily-polluted side of the ecotone- point (left) the community is composed of a few pollution tolerant opportunistic species. On the less polluted side of the ecotone-point (right) the community tends to approach that of an unpolluted site. The community at the ecotone point is a mixture of the two adjacent communities.

Figure 5.4 A generalised species abundance biomass (SAB) curve diagram adapted from Pearson and Rosenberg (1978).

At their simplest, benthic indices may be calculated from the ratios of the major zones within a system e.g. Jeffrey et al.. (1985) and Wilson (2003), but more precision is usually demanded. Consequently, benthic indices are more commonly based on the comparison of the sampled community (S, A and B severally or in combination) against a reference situation.

One of the most commonly used measures is the Shannon-Weiner index, influenced by both the number of species present and how evenly or unevenly species are distributed (Sanders, 1968) and which in fresh water has reached the status of an absolute quality index in that values of <3.0 can be taken to be polluted. However, in marine situations, Pearson and Rosenberg (1978) noted that the Shannon index could not produce a continuous trend along a pollution gradient and a particular problem for inshore marine use is that the value derived is dependent on the number of species in the community (since the maximum value of H' = logS: the various dominance indices (e.g. Simpson's

lambda (λ)) are based on similar premises. Rarefaction (Sanders 1968) has been considered a safer technique to use than the Shannon-Weiner index in an ecological context (Pearson and Rosenberg 1978).

Some control can be exerted over variability induced by sampling variation (e.g. different sample or grab sizes) through the technique of rarefaction (Sanders 1968). Rarefaction extrapolates the number of species distributed amongst a reduced number of individuals that have been derived from the original sample (Pearson and Rosenberg, 1978). It was devised by Sanders (1968) as simple method for measuring species diversity and can be used in both spatial and temporal measurements. This method is valid only when the same organisms sampled in the same manner (Magurran, 2004) can be compared and contrasted and will not work when the fauna is aggregated (Sanders, 1968). It should also be noted that this method does not depend on the number of species in a sample, but depends on the shape of the species abundance curve (Sanders, 1968).

Sanders (1968) noted a good agreement between rarefaction and Shannon-Weiner index but Pearson and Rosenberg (1978) believed that the rarefaction technique was both a better and a safer technique to use than the Shannon-Weiner index in an ecological context.

In fresh-water practice, these have been greatly augmented by the development of the Saprobic Index, in which a score is derived from the known pollution-tolerances of the species sampled and now widely used in the river invertebrate prediction and classification system (RIVPACS) package (Wright, 2000). A similar approach in marine coastal areas has been used to derive the ATZI marine biotic index (AMBI) and the IQI. From Grall and Glemarec (1997) and Glemarec and Hily (1981) who derived 5 different groups of species commonly found along an organic enrichment gradient (Table 5.4). A biotic index (BI) was derived with 8 distinct BI values, which made it possible to define the different stages of community degradation from 0 to 7

Group 1: species very sensitive to organic enrichment and present in normal conditions.
Group 2: species indifferent to enrichment, always present in low densities without significant variations in time.
Group 3: species tolerant to excess organic matter enrichment; these species may occur in normal conditions but their populations are stimulated by the organic enrichment.
Group 4: second order opportunistic species; these are small species with short life cycles, adapted to life in reduced sediment where they can proliferate.
Group 5: first order opportunistic species; these are deposit feeders that proliferate in reduced

Table 5.4 Benthic macrobenthic groupings along a gradient of pollution tolerance (from Grall and Glemarec 1997; Glemarec and Hily 1981).

Borja et al. (2000) noted that the original BI had a potential limitation in that each BI had a specific

value and so a formula was proposed to obtain a continuous BI from the percentage abundance of each species within each sample, known as the Biotic Coefficient (BC). It is similar to the BI in that the BC has values from 0 to 7 with BC=7 being azoic sediment.

$$BC = \{(0*\%G1)+(1.5*\%G2)+(3*\%G3)+(4.5*\%G4)+(6*\%G5)\}/100$$

This has been further refined into the AZTI Marine Biotic Index (AMBI) developed for the WFD to establish the ecological quality of European coastal waters.

The principal advantage of AMBI is that it is only affected by changes in impact source, not by changes in cyclic variations (ICES-CM, 2003). However, even though it is a robust method, the robustness deteriorates when samples containing very few species are found. There is also the probability that misclassification could occur by assigning species to one of the five groups (WFD).

Classification	Biotic Coefficient	Biotic Index	Dominant Ecol. Group	Benthic health
Unpolluted	**0.0 <BC < 0.2** **0.2 <BC < 1.2**	**0** **1**	**I** *Tellina tenuis*	**Normal** **Impoverished**
Slightly Polluted	**1.2 <BC < 3.3**	**2**	**III** *Neanthes (Nereis)*	**Unbalanced**
Meanly polluted	**3.3 <BC < 4.3** **4.3 <BC < 5.0**	**3** **4**	*Polydora, Pronospio* ***(IV)*** **IV - V**	**Transitional to slight pollution**
Heavily polluted	**5.0 <BC < 5.5** **5.5 <BC < 6.0**	**5** **6**	**V** *Capitella capitata*	**Transitional to heavy pollution**
Extremely polluted	**Azoic**	**7**	**Azoic**	**Azoic**

Table 5.5 AMBI categories along with examples of species and overall benthic health (From Borja et al. 2003).

5.4.2. Materials and Methods

In this project, only 3 of the 4 Tier II sites could be sampled: Dublin (5 sites, 5 samples/site); Wexford (4 sites, 5 samples/site); and Cork (2 sites, 5 samples/site). In all cases samples were taken with a 0.1m^2 van Veen grab, sieved through a 0.5mm mesh and preserved on board. Samples were sorted and species identified by the benthic monitoring team at the Marine Institute and Aquafact International Services Ltd. Physical character differences were accounted for with particle size analysis. The basic diversity indices were derived using PRIMER 6, and the infaunal trophic indices, AMBI and IQI, calculated with the routine supplied by the EPA.

5.4.3. Overall Findings and Conclusions

A summary of the results, along with selected indices is given in Appendix 6. It is immediately apparent that there is considerable variability, not just within the sample location, but even within the same site. For example at Wexford, site 2 (and to a lesser extent site 3) had both fewer individuals and fewer species (even adjusted for sample numbers by ES(100) but not sample 3 from this site) than the other Wexford sites. This sets Wexford apart from the other two locations. Omitting Wexford sites 2 and 3 from the comparison shows that most of the other samples fall within a fairly narrow range of the other metrics, e.g. the maximum H' value is 5.00 (Dublin 3-5) and the minimum 2.15 (Cork 2-2) with all three locations showing both high and low values. There was some difficulty running the AMBI/IQI routine, and some taxonomic judgements are called for in codifying the raw data. On the basis of the results presented here, it was impossible to distinguish among the three locations based on benthic indices alone. The merits and drawbacks of the various benthic indices are acknowledged, with particular emphasis on the difficulties in shallow or estuarine waters (see e.g. Gray 1979; Wilson 2003; Warwick et al. 2010). From the locations sampled in this project, it is evident that there can be major differences among sites at the same location and even a degree of within-site variability due in part to the difficulties of obtaining 'proper' sampling replicates This makes it difficult to accurately measure the health of the system.

As with all non-specific indices, what is measured is the community response to a number of variables. The AMBI/IQI does try to correct for the different sediment communities by incorporating sediment particle size, but that still leaves other environmental influences (e.g. salinity) as well as the very real possibility that the underlying model (Pearson and Rosenberg, 1978), drawn up largely on the basis of response to organic pollution, may not accurately reflect the community response to other types of contamination (see e.g. Rygg, 1986).

Since no single index worked 'better' than any of the others, it is recommended to continue with the PRIMER and AMBI routine.

6. Integrated assessment framework: Chapter six

6.1. Background on Integrated Assessment of Contaminant Impacts and Levels in Irish Waters

Development of a framework for integrating biological effects and chemical data is an essential component to enable successful integrated monitoring. The framework for integrated chemical and biological monitoring of contaminants was recommended by OSPAR MIME in 2011 and adapted by HASEC in 2012 to run on a 3 year trial basis. Important components in the system are fish, i.e. dab (*Limanda limanda*), flounder (*Platichthys flesus*), plaice (*Pleuronectes platessa*) and mussel (*Mytilus edulis*). An additional component of the integrated framework, mandatory in OSPAR CEMP, is imposex in the gastropod *Nucella lapillus*. Through a series of ICES/OSPAR workshops involving the study group for integrated monitoring of biological effects and contaminants (SGIMC), assessment criteria (Appendix 2) and guidelines have been developed for assessment of contaminant impacts in coastal and offshore areas. A multi-step process is proposed to allow evaluation of selected components (Figures 6.1-6.3 below). This approach follows on from experience of the assessment of contaminants data for sediment, fish and shellfish in OSPAR contexts. The water component currently only comprises hydrography (salinity, temperature), but may in the future also include passive sampling techniques and/or bioassay analyses of extracts or water.

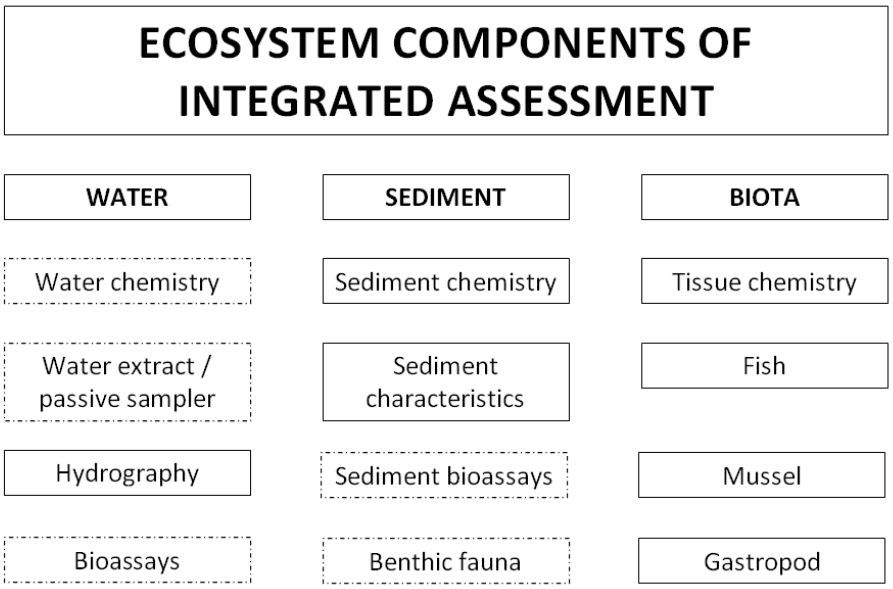


Figure 6.1 Components in an assessment of contaminant impacts in marine ecosystems; solid lines: compulsory, stippled lines: optional (ICES/OSPAR 2011; see also ICES, 2012).

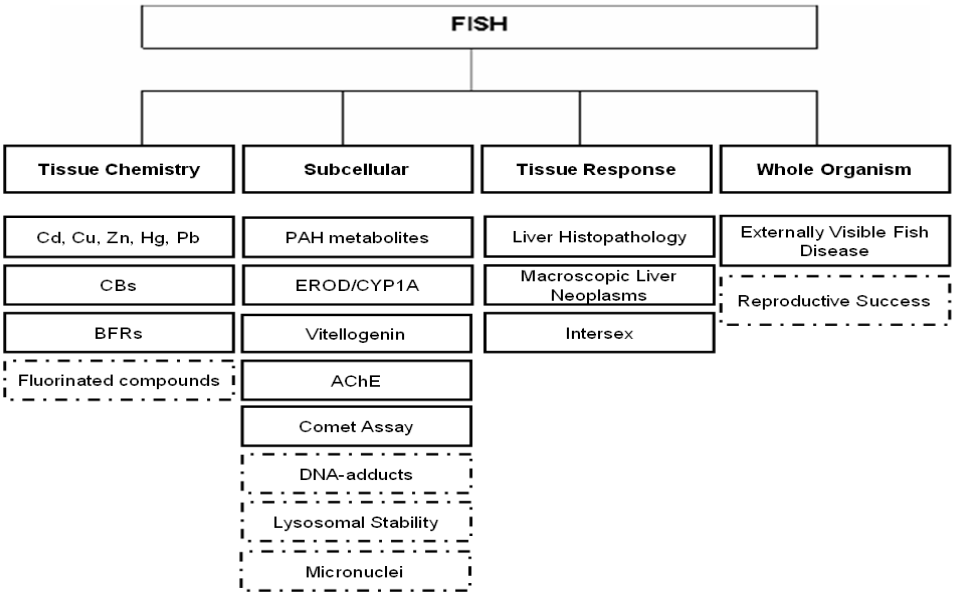


Figure 6.2 Components in an assessment of contaminant impacts on fish; solid lines: compulsory, stippled lines: optional (from ICES/OSPAR 2011; see also ICES, 2012).

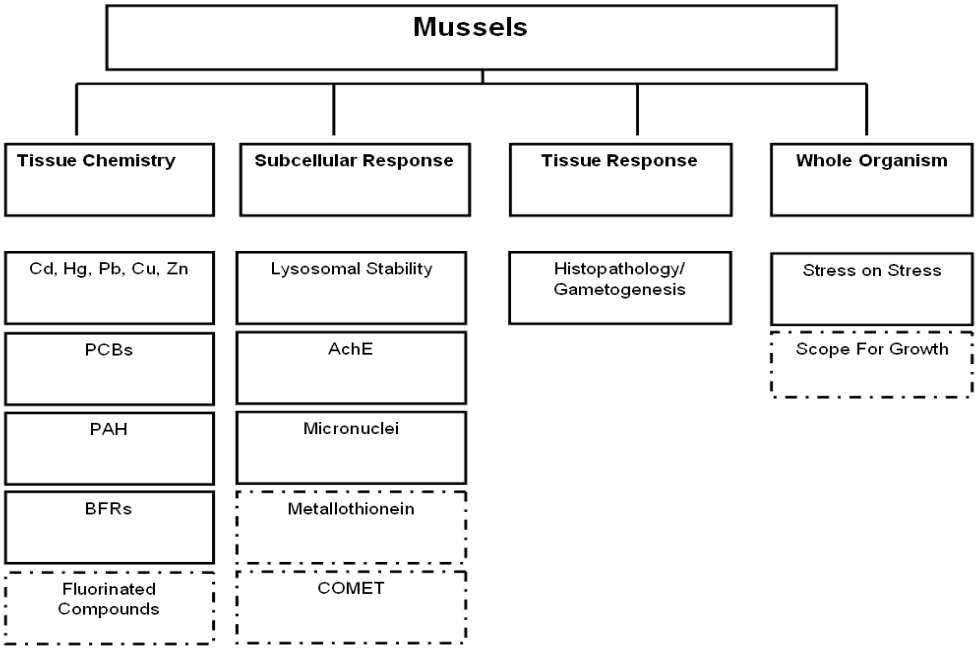


Figure 6.3 Components in an assessment of contaminant impacts on mussels; solid lines: compulsory, stippled lines: optional (from ICES/OSPAR 2011; see also ICES, 2012).

6.1.1. Methodology for Assessment Framework

This approach builds on OSPAR MON regional assessment tools developed for contaminants. Individual assessments of determinands (contaminant concentrations or biological responses) in specific matrices at individual sites are assessed against defined assessment criteria. The

assessment criteria suitable for use with chemical components of the framework include those used in the OSPAR Quality Status Report 2010, i.e. OSPAR BACs and EACs and EU EQSs; EC food safety regulation limits may be used where EACs or EQSs are not available (OSPAR, 2008). BACs and EACs for biological effects have been developed over recent years and are included in OSPAR background documents (ICES, 2012). Initial comparisons determine whether the determinand and site combinations are <BAC (blue), between the BAC and EAC/EC (green), or >EAC/EC (red); missing data are generally indicated using grey. This summarised indicator of status for each determinand can then be integrated over different levels: matrix (sediment, water, fish, mussel, gastropod), site and region, and expressed with varying levels of aggregation to graphically represent the proportion of different types of determinands (or for each determinand, sites within a region) exceeding either level of assessment criteria.

The integration of data can therefore be performed on multiple levels. Problem areas can be identified allowing possible identification of causative matrices responsible for where EACs are exceeded. Any stage of the assessment can be readily "unpacked" to a prior stage to identify either contaminant or effects measurements of potential concern or sites contributing to poor regional assessments. This allows the representation of monitoring data alongside contaminant data using a graphical representation approach. Both spatial (Tier I and Tier II for this present study) and temporal data obtained from Marine Institute (as outlined in methodology chapter) were used in this assessment.

For biological effects, there was no assessment criteria (BAC or EAC) for Vtg in dab and therefore Vtg is not included in the assessment. However, a "pilot scale" assessment using Vtg has been completed and is presented in the EDC case study. Other data not included due to insufficient numbers of samples or inadequate QC are fish disease index (external diseases) and MT in mussels. Some sediment ecotoxicology data were excluded due to lack of assessment criteria. These included LC/EC_{50} tests on porewater and elutriates with *Tisbe battagliai, Skeletonema costatum and Vibrio fischeri.* Data were included for the whole sediment *Arenicola marina* and *Corophium volutator* tests for which assessment criteria are established. Currently assessment criteria for PCBs/PAHs for passive sampling are at an early stage of development and therefore these results were not included in assessment. PS data for PAHs and PCBs at a number of Tier II generally support biomonitor species data. Data is available for a wide range of determinands however only determinands where both BAC and EAC were available were used for the assessment. The stepwise integration process is described below.

Step 1: Assessment of Monitoring Data by Matrix Against BAC and EAC

All determinands available were compiled and presented by monitoring matrix (sediment, water or biota) and expressed as a colour depending on whether the value exceeds BAC or EAC.

Step 2: Integration of Determinands by Matrix for a Given Site

For each of the matrices, the results of the individual determinand assessments are aggregated

into categories: contaminants, exposure indicators, effects indicators. Biological effects measurements are separated into different categories (exposure and effects) depending on whether an EAC-equivalent assessment criterion (AC) has been set or not. These categories have been termed 'exposure indicators' where an EAC has not been set and 'effects indicators' where an EAC (equivalent to significant pollution effect) has been set. Matrix integration and determinand category are expressed by tri-coloured bars, showing the proportions of determinands that exceed the BAC and EAC. For assessment purposes each contaminant, effect or exposure technique within each matrix carry equal weighting factors. Exceedence of contaminant or effects based EACs is indicative of potential significant detrimental effects to individuals or populations of marine organisms.

Step 3: Integration of Matrices for a Site Assessment

Matrices information is aggregated for a particular site and expressed by determinand category. Thus the outcome of assessment of all determinands from all matrices can be expressed for one site. For this approach the percentages of each colour in one column (e.g. contaminants) is summed for each matrix and the colour sums scaled to 100%. For this assessment this will be the highest level of aggregation. However, for assessments covering larger regional geographical areas where assessments need to be undertaken across multiple sites, a further level of integration is required (Steps 4 and 5).

Step 4: Regional Assessment across Multiple Sites

This can be done at multiple levels, i.e. aggregation of data at the sub-regional, regional and national levels, in different ways to express both the overall assessment of proportion of determinands (across all matrices) exceeding both assessment thresholds (BAC/EAC) and by determinand for the region showing the proportion of sites assessed in the region that exceed the thresholds. Both approaches show the overall proportion of determinand/site incidences of threshold exceedence.

Step 5: Overall Assessment

The assessment by region can be aggregated further into a single schematic showing the proportion all determinands across all sites that exceed BAC and EAC. This can be used for the purposes of an overall assessment. The overall assessment can be easily "unpacked" through the steps above to determine which sites and determinands (effects types or contaminants) are contributing to, for example, the proportion of red (greater than EAC) data, and thereby potentially leading to failure to achieve the desired status for a region.

6.1.2. Assessment by Matrix

Table 6.1 shows the assessment results by site for sediment determinands including contaminants and effects measurements. It is clearly presented where pressures are occurring for both contaminants and effects. It is clear that there are exceedences of EACs for some the contaminants

at 5/7 sites with the exception of Newquay and Shannon. However no exceedences were observed for sediment bioassays indicating that there is no contaminant related effects evident in these particular organisms. This type of assessment can be performed for both fish and mussels also whereby it is possible to look at overall contaminant levels and their potential effects.

Table 6.1 Assessment of sediment data against OSPAR/ICES assessment criteria (BAC / EAC) Grey cells = not analysed. Orange cells indicate not assessment criteria

Parameter	Tralee	Tolka	Cork	Kinvara	New Quay	Bantry	Shannon	Wexford
Cd								
Hg								
Pb								
As								
Cr								
Cu								
Ni								
Zn								
TBT								
Naphthalene								
Phenanthrene								
Anthracene								
DBT								
Fluoranthene								
Pyrene								
Benzo(a)anthracene								
Chrysene								
Benzo(a)pyrene								
Benzo(ghi)perylene								
Indeno(1,2,3-c,d)pyrene								
PCB 28								
PCB 52								
PCB 101								
PCB 105								
PCB 118								
PCB 138								
PCB 153								
PCB156								
PCB 180								

6.1.3. Assessment by Area

Data integration by matrix and site allows assessments to be made at a regional level for each of the different categories. Below data integration is presented with all available data for nine locations monitored around Ireland with different monitoring matrices integrated over the three categories contaminants, exposure and effects. Figure 6.4 shows the aggregated

assessment of contaminants, exposure and effects for nine locations in Irish waters. It is demonstrated that there are exceedences of EAC in both contaminants and effects for many of the sites monitored. Exposure markers consisting of EROD measurements only (male and female) were only available for four of the locations (Tier II sites). Exposure resulted in an exceedence at Dublin Bay. When unpacked it was determined that EROD in male plaice sampled from Dublin Bay had exceeded EAC. A number of exceedences are evident in Dublin Bay in all three categories (contaminants, exposure and effects) and therefore this location was further unpacked for assessment of matrices on a site by site basis. Figure 6.5 shows all determinands measured in Dublin Bay samples. It is evident that there are a number of exceedences in sediment and biota both in the contaminants, exposure and effects.

The framework mechanism has been shown to provide valuable summary information on the number of exceedances in relation to assessment criteria and potential problem area can be identified. Reporting of future datasets and ongoing development of assessment criteria should allow this to be further addressed and for spatial representivity toolkits to further evolve.

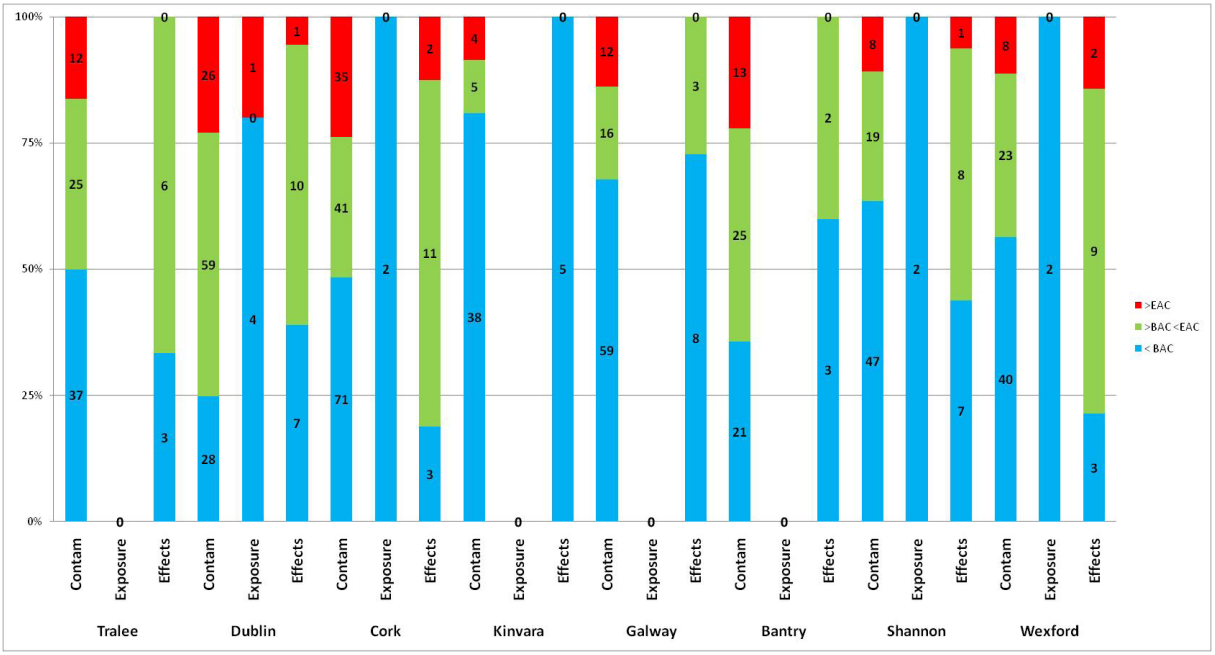


Figure 6.4 Assessment of contaminants, exposure and effects for eight locations in Irish marine waters

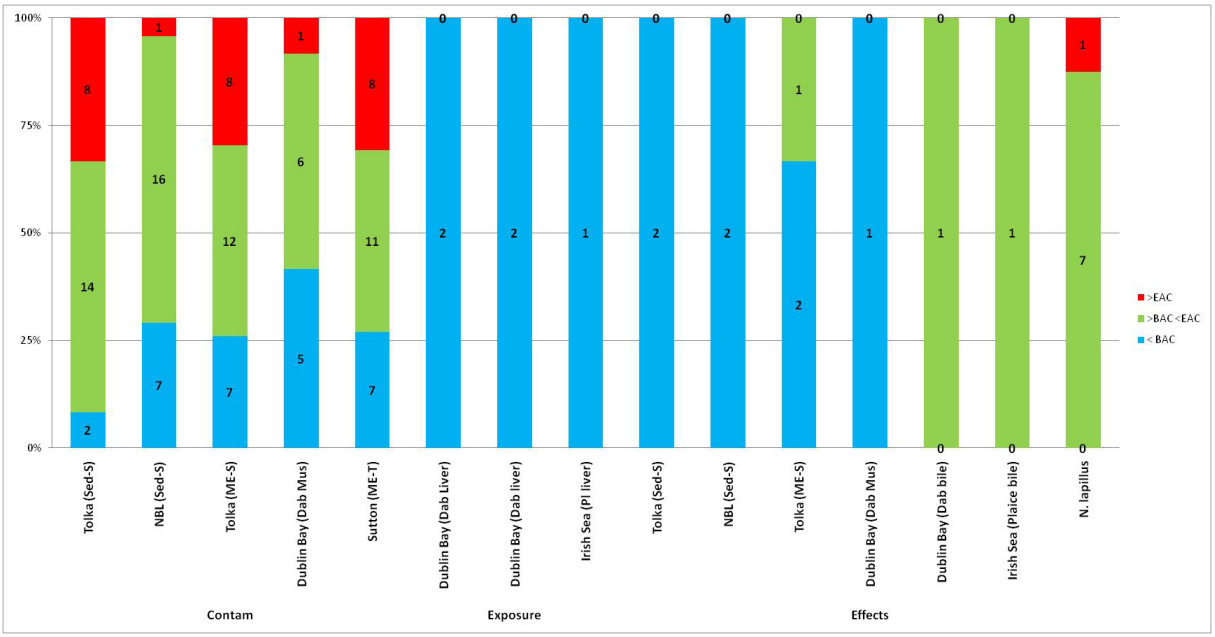


Figure 6.5 Assessment of contaminants, and exposure and effects biomarkers in all matrices at one area, Dublin Bay (Sed = sediment, ME = *Mytilus edulis*, S = spatial, T = MI temporal trend sample.

In addition to the ICES/OSPAR, (2011) recommended contaminants and biological effects, a number of other analyses were conducted on samples collected during the course of the Sea Change project (See appendix 5). However due to a lack of assessment criteria and/or background documents, these are not currently recommended for inclusion in the integrated framework.

The approach used for assessment is considered by ICES/OSPAR SGIMC as suitable for the determination of GES for Descriptor 8 under the MSFD. ICES/OSPAR SGIMC proposed GES is indicated if 95% of the data is less than EAC. However there are a number of requirements needed for determination of GES.

1. That there is a complete dataset available including all elements of the integrated framework consisting of all elements of the ecosystem approach. The scheme can be applied to datasets with missing data and determinands, but the validity of the assessment will decrease with increasing missing data.
2. The number of locations and type of sites are to be carefully considered for inclusion in the monitoring framework. For example one area can consist of a number of locations which may or may not be in the MSFD area. Extrapolation of status is not possible with limited sample numbers.
3. Confidence in assessment is essential and for future monitoring it is important to create a confidence correction for data where there are missing sample numbers.
4. Such an approach should be based as much as possible on the simultaneous measurement of physical properties/supporting factors such as temperature and salinity.

5. Assessment criteria are not available for all contaminants/effects measurements in all species and therefore monitoring should focus on selection of species where assessment criteria are available in order to prevent missing determinands.

6.2. Future Recommendations

This integrated assessment framework is proposed by ICES/OSPAR SGIMC for assessing compliance with proposed D8 GES criteria. For the purposes of the data produced for this project it was an ideal method for assessment of integrated biological and chemical effects and is recommended for future integrated monitoring assessments.

7. Evaluation of integrated monitoring and potential role in OSPAR/MSFD assessment: chapter seven

It is evident from the outcomes of this project that a weight of evidence approach as put forward by the study group on integrated monitoring of contaminants (ICE/OSPAR, 2011) can provide a more holistic elucidation of pollution effects at many organisational levels. However there are some difficulties with linking some biological effects techniques with chemical analysis. It is clearly evident from this project that individual biomarkers vary with sensitivity and specificity for contaminants and that no single biomarker alone can evaluate effects of pollution of mixtures of contaminants. Some biomarkers have greater potential than others for incorporation in monitoring based on efficacy as an accurate indicator of response to the pressure, complementarities within a suite of techniques, practicality and cost. Based on the experience of the project an evaluation was carried out to provide recommendations on preferred techniques for incorporation in a cost-effective integrated monitoring programme. Moreover, it is discussed below how this may fit into ongoing developments with respect to the MSFD and OSPAR objectives.

7.1. Evaluation of Suite of Biomarkers

The ICES/OSPAR, (2011) proposed biomarkers are useful only in combination with each other and each biomarker has strengths and weaknesses. The indicators proposed for MSFD requirements are concentration levels of contaminants and biological effects of contaminants where a cause and effect link has been established. It is clear that only one biomarker, imposex in marine gastropods, meets these requirements. In order to evaluate the range of biomarkers used in this project, an evaluation matrix for biomarkers is shown in Table 7.1. This takes into account the direct application of the biomarker to marine systems, how fast the answer is generated, how sensitive the response is to low contaminant concentrations, lack of false positives/negatives generated by the biomarker, specificity of response (general or exposure effect), degree of instrumentation and skill level required to carry out the analysis of the biomarker, cost benefit, whether there are assessment criteria available and whether there are quality assurance schemes available.

Project evaluation through expert judgement recommends the use of up to seven biomarkers including benthic monitoring, LMS, SFG, mussel histopathology, Vtg, AChE and imposex. These techniques cover all organisation levels and, when combined support the weight of evidence approach. These techniques are readily applicable to both temporal and spatial monitoring. For future monitoring with these techniques it is essential to establish seasonal background levels for monitoring species at lowly impacted sites in order to be able to use them effectively.

Table 7.1 Evaluation matrix of biomarkers and indices used for the Seachange project
Key: Realism-direct applicability to marine systems; Speed-how fast answer generated; Sensitivity-response to low contaminant concentration; Robustness-lack of false positive or negative; Specificity-

to target contaminant(s); Generality-generalised stress (incl. environmental) response; Technical-degree of instrumentation (beyond basic lab) required; Skill-level required by operatives to generate answer; Cost benefit-required to generate answer (excluding sampling costs);
Quality assurance available, A: BEQUALM, B: QUASIMEME, C: Between particular laboratories, D: No formal scheme available, E; Assessment criteria-whether assessment criteria is available (A) or missing (M); Evaluation-based on expert judgement taking into consideration all criteria.
Response type: Eco: Ecosystem; Sub: Subsellular; WO: Whole organism; Pop: population; Tis: Tissue
1=low, 5=high (e.g. 5 (high) is good for technical but not good for practical scores)

Biomarker	Response	Contaminant specificity	Realism	Sensitivity	Robustness	Specificity	Generality	QA	AC	Technical	Skill	Cost benefit	Speed	Evaluation
Benthic Monitoring[1]	Eco	GS	5	1	2	N	1	A	M	4	5	5	1	4
LMS-NRR (Mu)[2,3]	Sub	GS	1	5	3	N	1	C	A	1	2	1	5	4
SFG (Mu)[2,3]	Org	GS	4	3	4	N	2	D	A	3	2	2	3	4
SoS (Mu)[2,3]	Org	GS	4	2	3	N	2	D	A	1	1	1	4	3
Condition (Mu)	Org	GS *	4	1	2	N	1			2	1	1	5	
MT (Mu)[2]	Sub	Metals/GS	3	4	3	3	5	C	M	3	3	2	3	2
ALP (Mu)	Sub	EDCs	3	4	4	5	5	D	M	3	3	2	3	1
Sed Eco: *C. volutator* (WS)	Pop	GS	4	2	2	N	2	A	A	2	1	2	2	3
Sed Eco: *A. marina* (WS)	Pop	GS	4	2	2	N	2	A	A	2	1	2	2	3
Sed Eco: *T. battagliai* (P) and (EL)	Pop	GS	3	3	2	N	1	A	A	2	2	2	1	3
Sed Eco: *S. costatum* (P) and (EL)	Pop	GS	3	3	1	N	1	A	M	2	2	2	1	3
Sed Eco.: *V. fischeri* (P) and (EL)	Pop	GS	3	3	2	N	1	A	M	2	2	2	1	3
Mussel histopathology[2,3]	Tis	GS	5	5	5	N	3	D	A	4	4	4	4	4
Fish Path: External diseases (fish)[2,3]	Tis	GS	4	2	4	N	4	A	A	4	3	1	4	3
Fish Path: Internal diseases (fish)[2,3]	Tis	GS/ Carcinogenics	4	2	4	N	5	A	A	2	3	5	2	3
AChE (Mu/fish)[2,3]	Sub	Pesticides	1	5	2	3	2	C	A	3	3	3	4	3
EROD (fish)[2]	Sub	PAHs/PCBs	1	5	4	4	4	A	A	3	3	3	4	2
Bile metabolites (fish)[2,3]	Sub	PAHs	3	5	4	3	3	B	A	3	3	3	4	4
Imposex [2,3] (gastopods)	WO	TBT	4	5	4	4	5	B	A	1	3	1	4	5
Intersex (gastropods)	WO	TBT	4	3	3	4	5	D	M	1	3	1	4	4
Vtg (fish)[4]	Sub	EDCs	4	4	4	4	5	D	M	1	3	5	3	4
ER-LUC (Mu)		EDCs	3	5	4	4	5	D	M	5	3	3	3	4
Comet (Mu)[2]	Sub	Genotoxic	1	4	4	N	2	D	A	5	3	2	5	3

[1] WFD monitoring, [2]BAC available, [3]EAC available, [4]antibodies not freely available.

7.2. MSFD and OSPAR Objectives. How Can Integrated Monitoring Contribute to the Assessment Requirements of the European Legislation and Marine Conventions?

The WFD; Directive 2000/60/EC is the primary legislation in place requiring monitoring of pollutants discharged into river basins. The Directive requires Member States to develop and implement River Basin Management Plans to ensure *Good Chemical* and *Good Ecological Surface Water Status* is achieved and the Directive includes transitional and coastal water in its scope. The outer boundaries are territorial water limit (12 nautical miles from baseline) with respect to *Chemical Status* i.e. compliance with community-wide EQS set in EC legislation, and 1 nautical mile from baseline for *Ecological Status* which includes compliance of concentrations of other relevant pollutants with national standards. Community-wide EQS have been set for priority substances and priority hazardous substances for dissolved water phase (cadmium, lead and nickel), total water (specific 35 organic substances) and biota/prey tissue (mercury, HCB and HCBD) under the WFD. An amended Directive including new EQS is imminent. These EQS and additional national EQS for other relevant pollutants are enacted in Irish Statutory Instruments SI 272 of 2009 as amended by SI 327 of 2012. The primary focus of contaminant monitoring for WFD is assessing compliance with concentration based standards for water and to a lesser extent biota. Biological effects monitoring has no clear role, although it could be potentially used in investigative monitoring.

The MSFD requires that *concentrations of contaminants are at levels not giving rise to pollution effects* as one of 11 qualitative descriptors of GES (Descriptor 8). The Directive requires member states to define GES including Targets and Indicators for their marine waters by 2012 (articles 9 and 10) and establish monitoring programmes by 2014 (article 11). Moreover Commission Decision 477/2010/EU lists the following criteria and related indicators for Descriptor 8

8.1. Concentration of Contaminants

— Concentration of the contaminants mentioned above, measured in the relevant matrix (such as biota, sediment and water) in a way that ensures comparability with the assessments under Directive 2000/60/EC (8.1.1)

8.2. Effects of Contaminants

— Levels of pollution effects on the ecosystem components concerned, having regard to the selected biological processes and taxonomic groups where a cause/effect relationship has been established and needs to be monitored (8.2.1)

— Occurrence, origin (where possible), extent of significant acute pollution events (e.g. slicks from oil and oil products) and their impact on biota physically affected by this pollution (8.2.2).

This decision requires that "the presence of contaminants in the marine environment and their biological effects are kept within acceptable limits, so as to ensure that there are no significant impacts on or risk to the marine environment". There is a clear overlap between the WFD and

the MSFD (latter includes coastal waters) and there is a need for coherent implementation of these directives, especially as the pollution risk from waterborne priority substances is greater in inshore waters than offshore. Compliance monitoring of concentrations with EQS/EACs will be a key element of indicator 8.1 but trend monitoring in marine matrices (biota, sediment and water) as implemented through OSPAR CEMP will also be important as the aim is to assess progress towards GES in response to measures taken.

Indicator 8.2.1 requires assessment of pollution effects on ecosystem components at various levels of biological organisation (JRC). Given the uncertainty associated with many individual effects techniques and the difficulty in unambiguously relating them to contaminant pressure the use of individual biological effect techniques as standalone indicator/targets to define GES (e.g. similar to WFD application of EQS) may be problematic. The exception to this is reproductive impairment in gastropod molluscs that is both specific and sensitive and can be clearly linked to organotin contamination. Consequently, with specific reference to OSPAR criteria for imposex in dogwhelks (*Nucella lapillus*), this is presently the only biological effect target and associated indicator (8.2.1) put forward by Ireland under article 10 of the MSFD (http://cdr.eionet.europa.eu/ie/eu/msfd8910/acsie/envuwsbg). This is a limited approach to the use of biological effects tools to assess pollution effects. While we do not recommend adding additional specific biological effects as "stand alone" indicators/targets for MSFD purpose, using a greater suite of biological effects in an integrated biological effects-concentrations indicator may give a better overview of ecosystem health. This would be supplementary to specific contaminant (EQS) and imposex targets. It remains to be seen how an indicator that straddles Descriptor 8 criteria will fit within MSFD reporting fields but the integrated approach provides a method for aggregating across indicators to provide an overall assessment of GES for Descriptor 8.

OSPAR has been central to developing the approach in integrated contaminants monitoring and assessment. How this will be taken forward within the CEMP may ultimately depend on how Contracting Parties see this fitting into the MSFD. OSPAR are currently piloting the integrated monitoring approach within the CEMP framework through the MIME working group this should also provide further guidance for incorporation into the national assessment of GES for Descriptor 8. It is recommended that the output and data from this project are fed into this OSPAR pilot project.

7.3. Future Roles for Integrated Monitoring

Incorporating the suite of biomarkers/biological indicators alongside monitoring of concentrations in water, sediments and biota is suggested as a way forward. However, aside from imposex, it is not recommended that the biological effects tools have "stand alone" specific targets but that GES could be defined by the integrated indicator. Contaminants biomonitoring should continue to include shellfish (bivalve molluscs) in coastal water but also include fish in accordance with the OSPAR guidelines. Seabird eggs are also potentially a very effective matrix as an indicator for contaminants

in higher trophic levels and across wide spatial scales with correct species selection. There is an OSPAR EcoQO for hazardous substances in seabird eggs (OSPAR 2010). Passive sampling is widely recognised as being a very important development for monitoring time-integrated bioavailable fractions of non-polar organics and PDMS has been successfully used in this (section 5.3) and other studies at the Marine Institute.

Further consideration of how an integrated approach should be implemented is required. However, such a programme could incorporate the following:-

1. Contaminant monitoring in water, biota, sediment and passive sampling, trend assessments and compliance with EQS /EAC targets (MSFD Indicator 8.1.1 to assess specific targets)
2. Monitoring of imposex in dogwhelks to assess trends and compliance with OSPAR thresholds and MSFD Indicator 8.2.1 to assess specific target
3. Monitoring of a further suite of recommended biomarkers/biological parameters (benthic monitoring, LMS, SFG, mussel histopathology, Vtg and AChE). Results integrated with 1 and 2 to provide an integrated assessment to assess GES at MSFD Descriptor 8 level)

Spatial scales:

Biomarkers/biological effects tools can be used as screening methods to support identification of areas at risk as applied in Phase 1 of this project. The full integrated approach is best used for assessment of areas at highest risk (areas subject to greatest pressures or identified through chemical monitoring or biomarker screening as potential problem areas) given the costs. The application of the suite of biomarkers at a broader spatial scale than the one used in this project is essential in future monitoring.

8. Discussion and Conclusions: Chapter Eight

This project provides the first quality assured "baseline" information with respect to integration of chemical measurements and biological effects for Irish marine waters. The assessment mechanism and monitoring tools may support the determination of GES for Descriptor 8 under the MSFD. In particular biological effect tools with a potential role in defining GES and targets and indicators with respect to Criterion 8.2 (effects of contaminants) and the integrated approach provides a potential mechanism for assessing GES at Descriptor 8 level.

Biological effects and contaminant data from this project further support the conclusion that the quality of Irish transitional and coastal waters is generally good and in general where contaminant temporal trend data are available these are tending to be in a downward direction (McGovern et al., 2011).

Aggregating the assessments as conservative worst-case scenarios across parameters indicated that few areas exhibit parameters that are flagged with a less-than good (red) status and with other than low confidence assigned. More work is needed to establish whether clear effects are measurable in the more highly industrialized estuaries (Dublin, Cork and Wexford) which showed some indications of a greater degree of contaminant pressures and biological effects.

Toxicants are shown to have effects at the level of the individual and at cellular/molecular levels in molluscs and in some cases in fish. Further investigation into links between individual effects and top level indicators of ecosystem health such as fish pathologies and benthic community changes is warranted. It is apparent that the deleterious biological effect responses were not always correlated with levels of contaminants that were measured in the study.

There are a number of key reasons for this disconnect, including the intrinsic variability within the assays and the fact that the number of variables that can be controlled decreases with scale. In time, the former can be quantified and reduced with more data and suitable statistics, while the second is the primary reason for completion of integrated monitoring.

The future application of passive sampling techniques as a complementary technique to conventional water and/or biomonitor species analysis has been demonstrated to be a cost effective means of generating trace level dissolved water concentration data for primarily organic contaminants. In particular it has promise for reducing temporal variability and for contaminant selectivity through choice of membrane. The initial stages of development of assessment criteria are currently underway and the use of PS techniques is strongly supported within ICES/OSPAR. It is recommended that passive sampling should continue to form a core part of future Irish integrated monitoring programs to support integrated assessments and in support of WFD and MSFD monitoring commitments.

This project reports the first marine focused EDC chemical and biological effects dataset for Irish marine waters. Levels and effects reported in this limited pilot study are generally low, either at or below reference values such that often pristine areas could not be distinguished from those closer to anthropogenic pressures. Combined chemical and biological effects measurements suggest that further monitoring of steroid and EDC levels in water and ED effects in resident species may be merited in areas such as Dublin Bay and Wexford. Chemical and biological analyses were found to be complementary cost effective tools for the investigation of EDCs and ED in the marine environment. The most effective biological effect tools were the most selective (e.g. imposex, Vtg and ALP), possibly because they are less affected by other, non-controlled, variables.

Levels of imposex in dogwhelks determined at 12 locations show an ongoing improvement with the majority of the sites sampled meeting the OSPAR EcoQO, This reflects the now global prohibition on TBT as a marine antifoulant. Nonetheless, a number of areas still exhibit the effects of TBT contamination and, of the locations sampled, only in Galway Bay, Shannon and Tralee Bay did all sites meet the EcoQO. High levels of imposex (VDSI stages 5 and 6) indicative of sterility in females, were found at Castletownbere (all sites), Waterford (3/4 sites), Cork harbour (5/8 sites), Tralee Bay (1/4 sites) and Killybegs (1/7) sites. For the MSFD, imposex in dogwhelks is one of the key biological effects tools for investigation of Good Environmental Status in Descriptor 8. Overall however, levels around the coast even if >BAC were generally <EAC and the survey showed few sites of concern. Despite the significant downward trend in impact from TBT it is important that areas in the 2010/2011 study that showed non compliance with the EcoQO continue to be monitored in the future.

The reported data are invaluable for ongoing derivation of "fit for purpose" assessment criteria for marine waters. Such data have been lacking in the past and are key to enabling effective status assessment.

The ICES and OSPAR "integration" mechanism used provides summary information on the number of exceedances of assessment criteria however given the summary nature of the output it is not currently possible to include information in relation to contaminant levels. The development of EACs (or equivalents) and BACs needs to continue, this should allow further development of summary assessments and potentially incorporation of contaminant loading indices into outputs.

The results clearly indicate that chemical monitoring alone is not sufficient, but rather that it should be used selectively in trend monitoring and as a back-up to the biological effects to optimise the cost/benefit ratio of integrated monitoring. Continued research and monitoring is required in order to deliver further dedicated chemical measurement and ecotoxicological response datasets which are essential to enable derivation of scientifically sound and measurable, "marine endpoint" EACs which are key to supporting integrated assessments. Such tools are required in order to support management actions to limit/prevent risk to the environment.

A coordinated approach to the use of assessment thresholds in coastal and marine waters would result in a more comprehensive assessment of the impact of chemical contaminants and assist in ensuring that appropriate management measures be taken. OSPAR in conjunction with the EU will continue to develop improved assessment criteria for marine chemical monitoring. Contracting Parties will thus need to continue to undertake and develop monitoring under its CEMP with these programs also focusing where possible on further supporting the purposes of the WFD and MSFD. It may be possible to combine some monitoring to support both the WFD and the MSFD, or to extend the same rationale for the MFSD in order to promote sustainable development through the MSFD objectives.

It is only through wider scale continuance of "integrated" research and monitoring efforts that issues of spatial representivity, development of appropriate assessment criteria, development of quality assurance programs and statistical toolkits can be adequately addressed in the future.

Further research is required in the area of "combined effects" as a result of exposure of organisms to contaminant mixtures. The aquatic environmental risk assessment of chemicals and regulation against criteria such as EQS are inherently imperfect procedures which should be supplemented with toxicity assessment data.

In a wider context continued delivery of dedicated chemical measurement and ecotoxicological response information is essential to derivation of scientifically sound and measurable EACs which are key to supporting integrated assessments and to support management action required to limit/ prevent risk to the environment. Continuity in sampling design and consistency in data quality and analysis should be paramount in respect of future integrated marine monitoring programmes especially where the program has temporal components,

Ongoing generation of appropriate integrated dataset information will support future generation of relevant "marine endpoint" assessment criteria. There is a need to continue to deliver toxicological information for chemicals and a concurrent need to improve analytical methods to achieve lower limits of detection. Continuation of monitoring programs to support criteria development will as a consequence greatly improve the quality of environmental assessments.

It is recommended that mandatory elements of the CEMP for which appropriate assessment criteria are available form the basis for future "integrated" monitoring. This should include PCBs, PAHs, TBT and metals as a standard suite, this being supported where at all possible by the analysis of compounds included in the OSPAR pre-CEMP listing. These parameters will provide the focus for ongoing OSPAR initiatives related to generation of marine based assessment criteria and spatial representivity toolkits.

The data provided here form a basis for the integrated management of Irish coastal waters. They point clearly to those few areas and substances which might be of concern. The data also highlight, though the TBT example, the clear and obvious benefits that arise from prompt and decisive action arising from an integrated monitoring programme.

Conclusions

- An integrated monitoring programme (combining both chemical measurement and biological effects) is required as the most cost-effective support for sustainable management.
- The weight-of-evidence approach is recommended for generalist indicators, but a single failure may be appropriate for specific biomarkers (e.g. imposex).The impacts are generally low around the Irish coast. Sample analysis shows that most target substances (organochlorine pesticides, PCBs and PAH) were detectable with higher concentrations typically at locations subject to greater pressures. Exceedences of OSPAR EACs were generally restricted to some metals and in particular to other ubiquitous pollutants such as PCBs where in the example of CB118, a very low EAC exists. It is noted that for some of the historical persistent organochlorine contaminants that have been phased out, such as PCBs and organochlorine pesticides, there is some evidence for downward trends.
- More precision may be gained and value added to the monitoring programme through a) a coordinated and on-going synthesis of data to establish and improve reference values; b) development of response models (contaminants and environment) for biomarkers and c) refinement of biomarkers through genetic or proteomic approaches.

Glossary of terms

AA-EQS	Annual average environmental quality standard
AC	Assessment Criteria
AChE	Acetylcholinesterase
ALP	Alkali Labile Phosphate
AMBI	ATZI Marine Biotic Index
BAC	Background Assessment Criteria
BC	Biotic coefficient
BCF	Bio-concentration factor
BEQUALM	Biological Effects Quality Assurance in Monitoring
BI	Biotic index
BOD	Biochemical oxygen demand
BN	Benign neoplasm
BFR	Brominated Flame Retardent
CEFAS	Centre for Environment, Fisheries and Aquaculture (CEFAS)
CEMP	Co-ordinated Environmental Monitoring Programme
CF	Condition Factor
COMET	Assay used to measure genotoxic damage
CR	Clearance rate
DNA	Deoxyribonucleic acid
E1	Estrone
E2	17β-estradiol
EE2	17α-ethynylestradiol
EAC	Environmental Assessment Criteria
EcoQO	Ecological quality objective
EDC	Endocrine disrupting compound
EPA	Environmental Protection Agency
EQS	Environmental Quality Standard
Eutroph	Eutrophication
EROD	7-ethoxyresorufin O-deethylase
ER-LUC	Estrogen receptor mediated luciferase reporter gene system

FCA	Foci of cellular alteration
FRAP	Ferric Reducing Ability of Plasma
GES	Good Environmental Status
HASEC	Hazardous Substances and Eutrophication Committee
HCB	Hexachlorobenzene
HCBD	Hexachlorobutadiene
ICES	International Council for Exploration of the Sea
IQI	Infaunal quality index
ISO	International organisation for standardisation
JAMP	Joint assessment monitoring programme
LOQ	Limit of Quantification
LMS	Lysosomal membrane stability
MAC-QS	Maximum allowable concentration quality standard
MI	Marine Institute
MIME	Working Group on Monitoring and on Trends and Effects of Substances in the Marine Environment
MFO	Mixed function oxygenase
MN	Malignant neoplasm
MSFD	Marine Strategy Framework Directive
MT	Metallothionein
NNT	Non-neoplastic toxicopathic
NP	Nonylphenol
NSI	Non-specific inflammatory
NRR	Neutral red retention
OCP	Organochlorine Pesticide
OP	Octylphenol
OSPAR	Oslo and Paris Commission
QA	Quality assurance
PAH	Polycyclic aromatic hydrocarbon
PCA	Principal components analysis
PCB	Polychlorinated biphenyls
PDMS	Polydimethylsiloxane

Pharm	Pharmaceutical
POCIS	Polar organic chemical integrative sampler
POP	Persistent organic pollutant
PS	Passive sampling
QUASIMEME	Quality Assurance for Marine Environmental Monitoring in Europe
RESC	Radiation and Environmental Science Centre
RIVPACS	The River Invertebrate Prediction and Classification System
RPSI	Relative Penis Size Index
RR	Respiration rate
SATL	Shannon Aquatic Toxicity Laboratory
SFG	Scope for growth
SFPA	Sea Fisheries Protection Authority
SGIMC	Study group for integrated monitoring of biological effects and contaminants
SOS	Stress on stress
TCD	Trinity College Dublin
TBT	Tributyltin
VDSI	Vas deferens sequence index
Vtg	Vitellogenin
WFD	Water Framework Directive
WGBEC	Working group on biological effects of contaminants
WKIMC	Workshop on Assessment Criteria for Biological Effects Measurements
WP	Work package
WWTPE	Waste water treatment plant effluent
YES	Yeast estrogen screen

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Appendix I: List of publications and additional student projects

The following publications (Papers 1-8) directly emerged from the core work/deliverables performed during the course of this project. Paper 9 is a combination of results from previous imposex surveys performed by Dr. Dan Minchin and the imposex survey performed within the remit of this project. A considerable amount of additional work has been taken on through student moderatorship and MSc. projects (listed below) which allowed for extra data and coverage in addition to the core work package deliverables. One PhD dissertation (Ronan, 2013) and one MSc. dissertation (Rochford, 2012) arose directly as a result of this project and consisted mainly of the core work outlined in the project.

Publication list

Paper 1**: Assessment of biomarkers in *Mytilus edulis* to determine Good Environmental Status for implementation of MSFD in Ireland (2013)**

Giltrap, M., Ronan, J., Hardenberg, S., Parkes, G., McHugh, B., McGovern, E. Wilson, J.G.

Marine Pollution Bulletin, 71, 240-249

Paper 2: **A sensitive liquid chromatography tandem mass spectrometry method for the determination of natural and synthetic steroid estrogens in seawater and marine biota, with a focus on proposed Water Framework Directive Environmental Quality Standards (2013)**

Ronan, J. and McHugh, B. Rapid Communications in Mass Spectrometry 2013, 27, 738–746

Paper 3: **An integrated chemical and biological approach to investigating estrogenic contamination and endocrine disruption in the Irish marine environment** (in draft)

Jenny Ronan, Brendan McHugh, Michelle Giltrap, Heather Rochford, Rónán Mag Aoidh, James G. Wilson and Evin McGovern

Paper 4: **Acute exposure effects of 17α ethynylestradiol in *Mytilus* spp.** (in draft)

Jenny Ronan, Andrea Lenderink, Liam Curran, Michelle Giltrap, Silvia Hardenberg, Rónán Mag Aoidh, James Wilson, Evin McGovern and Brendan McHugh

Paper 5: **A multi-battery, multiphase two tiered approach for toxicity assessment of Irish marine sediment** (in draft)

Michelle Giltrap, Robert Hernan, Kathleen O 'Rourke, Jenny Ronan, Brendan McHugh, Evin McGovern and James Wilson.

Paper 6: **Mussel histology is a phenotypic anchor for biomarker data** (in review)

Michelle Giltrap, John Bignell, Jenny Ronan ,Colby Tanner, Rónán Mag Aoidh, Francis X O' Beirn, Brendan McHugh, Evin McGovern, James Wilson

Paper 7: **Fish histopathology, EROD and bile metabolites in Dab sampled from Irish coastal waters** (in draft)

Michelle Giltrap, Jenny Ronan, Neil Ruane, Evelyn Collins, Brett Lyons, Grant Stentiford, Brendan McHugh, Evin McGovern, James Wilson

Paper 8: **Integrated indices: A case study in Ireland**

Michelle Giltrap, Brendan McHugh, Evin McGovern, James Wilson

Paper 9: **Imposex in dogwhelks (*Nucella lapillus*) around the Irish coast: status and trend** (in review)

James Wilson, Dan Minchin, Evin McGovern, Brendan McHugh and Michelle Giltrap

List of student moderatorship projects completed:

Age classification and growth rates in plaice, dab and flounder species in Irish marine waters
Lorraine Bull, 2012.

The effect of parasite prevalence on scope for growth in Mytilus edulis
Miriam Whittle, 2012.

Endocrine disruption in Dublin Bay dab Limanda limanda
Ian McLoughlin, 2012.

Intersex in Littorina Littorea for selected locations around the Irish coast
Sarah Ebrill, 2012.

The effect of pollution status and parasite prevalence on the health status of Mytilus edulis.
Sean Kelly, 2011.

The Pollution Status of Castletown Estuary using BQI, PLI and Mytilus edulis as a bioindicator
Claire Frances Byrne, 2011.

Effects of maturity status on biochemical measurements in the blue mussel, Mytilus edulis.
Ciara Una Quill, 2010.

Effects of TBT in Dublin Bay using Nucella lapillus as an indicator
Cathy Maguire, 2010.

MSc projects:

Evaluation of the pollution status of three sites in Dublin Bay using a multi-biomarker approach with Mytilus edulis as a bioindicator.
Linda Charlotte Daniels, 2009.

The use of a multibiomarker approach for pollution assessment of caged mussels in Dublin Bay
Brian Boylan, 2010.

Endocrine disruption in Dublin Bay using Mytilus edulis as an indicator
Andrea Lenderink, 2010.

Biological Effects of Pollution in the Irish Marine Environment
Heather *Rochford*, 2012.

PhD project

PhD project

An integrated assessment of estrogenic endocrine disruption in the Irish marine environment, with particular focus on chemical measurements.
Jenny Ronan, 2013.

UREKA projects:

A preliminary study for the assessment of pesticide exposure around Dublin Bay using Mytilus edulis as an indicator.
Cathy Maguire, 2009.

Parasites in the blue mussel, Mytilus edulis in two case study sites in Dublin Bay.
Zara Cleere, 2009.

UREKA projects:

A preliminary study for the assessment of pesticide exposure around Dublin Bay using Mytilus edulis as an indicator.

Cathy Maguire 2009: UREKA project

Parasites in the Blue mussel, *Mytilus edulis* in two case study sites in Dublin Bay

Zara Cleere 2009 UREKA project

APPENDIX 2: DERIVATION AND APPLICATION OF ASSESSMENT CRITERIA FOR INTEGRATED MONITORING

Assessments of monitoring data for hazardous substances in the environment require relevant assessment tools. Data assessment compiled in this report has been completed using a combination of recognized criteria (OSPAR/ICES based) and those compiled solely for the purposes of the "pilot" EDC assessment. The derivation of both of these groupings is summarised below.

CEMP Based Assessment Criteria

For the OSPAR CEMP, assessment criteria for the hazardous substances analysed in marine sediments and biota have been developed. These include polycyclic aromatic hydrocarbons (PAHs), chlorobiphenyls (CBs) and the metals mercury, cadmium and lead. Assessment criteria are needed that relate to the key thematic questions set out in the OSPAR Joint Assessment and Monitoring Programme for hazardous substances, *i.e.*:

- What are the concentrations in the marine environment, and the effects, of the substances on the OSPAR List of chemicals for priority action ("priority chemicals")? Are they at, or approaching, background levels for naturally occurring substances and close to zero for man-made substances?
- Are there any problems emerging related to the presence of hazardous substances in the marine environment? In particular, are any unintended/unacceptable biological responses, or unintended/unacceptable levels of such responses, being caused by exposure to hazardous substances?
- For summarising and presenting assessments in a visual and meaningful way colour-based classification systems can be used based on the agreed assessment criteria.

A common understanding of the meaning of a classification scheme was developed in order to support a consistent use of transition points/colours in the presentation of assessments across matrices and contaminants. This common approach then allowed CEMP Assessment Criteria to be applied for the QSR 2010, the same assessment approach undertaken for the QSR 2010 was utilised in this current assessment.

Derivation of Biological Effects Assessment Criteria

Biological effects assessment criteria have been developed over recent years primarily through the ongoing work of the ICES/OSPAR SGIMC working group which has collated key background documents, assessment criteria, and quality assurance procedures for biological effects measurements. These background documents include assessment criteria in the form of background responses (for all methods) and EAC-analogues (where appropriate). The documents also generally include sections on quality control, and on the availability of external QA. These documents therefore meet the requirements for methods to become available for adoption into the CEMP. Values of the assessment criteria for biological effects measurements are tabulated below.

Considerations for a 'Traffic Light' Assessment Tool

In a wider context as this OSPAR approach is being tailored to support MSFD in that the use of transition points/colours may be of relevance to environmental status classification. The use of "green" has a relationship to "Good Environmental Status" to the extent that it is currently possible to assess this. The basic principle is that the transition from red to green implies a transition from an unacceptable risk to a state which is acceptable and where there is little or no risk. The interpretation of the proposed blue/green/red scheme in relation to hazardous substances and the type of management activity which may be possible for each colour is summarised in Figure A2.1

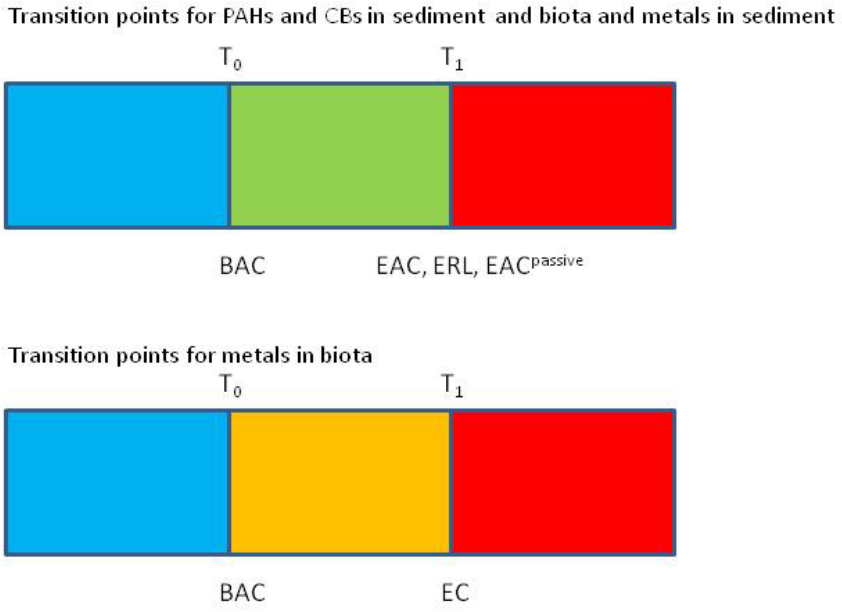


Figure A2.1: Traffic light system and the relevant transition point criteria for: a) PAHs and CBs in sediment and biota and metals in sediments, and b) metals in biota. The green/red boundary corresponds to the achievement of a statutory target (c.f. EQS in WFD terms) or a policy objective (e.g. EAC in OSPAR terms).

Summary Assessment Procedure Using the "Traffic-Light" Approach

The three colour traffic light system used for assessing hazardous substances data for marine sediments and biota was similar to that used for QSR 2010. The initial assessment of data was made in relation to a green/red or amber/red transition. A green assessment for a particular contaminant means that the environmental concentrations meet relevant statutory limits or policy objectives, and are satisfactory in that they present little or no risk. A red assessment means that the relevant limit or objective had not been met. The statistical aspects of the comparisons are on a precautionary basis. To report against the ultimate aim of the OSPAR Hazardous Substances Strategy that concentrations should be at, or close to, background concentrations, a second comparison has been made for a blue/green or blue/amber transition, against the relevant BAC. Concentrations that are significantly below the BAC, i.e. the OSPAR ultimate aim has been achieved, have been coloured blue. Concentrations that did not meet this precautionary statistical test remain green or amber.

Details procedures in relation to data treatment, normalisation and statistical tools as employed in the QSR and in this assessment report are presented elsewhere. http://www.ospar.org/documents/dbase/publications/p00461_background%20doc%20cemp_assessmt%20criteria_haz_subs.pdf

Derivation of EDC Assessment Criteria and Confidence Assessment

Classification was conducted by assessing data against relevant available assessment criteria to assign likely low ED risk status/less than good ED risk status. A lower confidence estimate was assigned if where sample numbers were low, or where there was lower confidence in the data or in the available assessment criteria. Assessment criteria are not readily available for a range of EDC related chemical and biological based measurements therefore this assessment collated definitive environmental protection and/or ecotoxicological based criteria (e.g. EQS) in addition to values accepted at national level and/or available in peer reviewed literature. As such it is accepted that the criteria utilised (see Table A2.4) may thus have associated lower levels of confidence and this is reflected in the assessment approach. The classification scheme used is outlined below:

	Low risk ED status		
Does not exceed definitive criteria[1]			Does not exceed definitive criteria but with low data confidence or does not exceed criteria which are of lower acceptibility[2].
Less than good ED risk status			
Exceeds definitive criteria[3]			Exceeds criteria but with lower confidence level or exceeds criteria which are of lower acceptability[4]

[1] Definitive criteria e.g. EQS value

[2] Does not exceed definitive criteria but with low data confidence (e.g. low sample numbers) and/or does not exceed criteria which are of lower acceptability (e.g. ER-LUC where only literature reference criteria and/or endogenous interference may be applicable.

[3] Exceeds definitive criteria such as EQS.

[4] Exceeds definitive criteria but with low data confidence (e.g. low sample numbers) and/or exceeds criteria which are of lower acceptability (e.g. ER-LUC where only literature reference criteria and/or endogenous interference may be applicable.

Where defined, WFD assessment criteria such as environmental quality standards (EQS) for other surface waters and quality standards (QS) for concentrations in biota used in the derivation of the EQS values were used. As E2 and EE2 are currently under review for inclusion in the WFD priority pollutant list, the provisional EQS values were used. Where maximum allowable concentrations (MAC) values have not been defined for a compound, the annual average (AA) EQS values are considered protective against short-term pollution peaks in continuous discharges since they are significantly lower than the values derived on the basis of acute toxicity. No legislative criteria were available for E1 however its potency relevant to that of E2 has been determined as 0.5 using the yeast estrogen screen (YES), (Routledge and Sumpter, 1997).

POCIS concentrations are reported in ng per device thus POCIS was used as a screening device, where non-detection corresponded to 'good' status and detection corresponded to 'present but no criteria available'. No definitive BAC is available for Vtg in plaice and dab, so the BAC for the closely related flounder was used as an indicative criterion. Definitive pass/fail criteria are not available for ER-LUC and ALP as natural variability in response may occur under different local and regional conditions, differences in impacts, adaptability of native species to local conditions and due to differences in organism gonadal stage. Caveats and transition protocols associated with individual criteria are detailed in Table A2.4.

Table A2.1: OSPAR CEMP derived assessment criteria as used in this report. All dry weight with the exception of for fish (wet weight).

	Sediment			Mussels					
	BAC	EAC	ERL	BAC	EAC	EC	BAC	EAC	EC
Cd	310		1200	960		5882	26		1000
Hg	70		150	90		2941	35		500
Pb	38000		47000	1300		8824	26		1500
As	25000		---						
Cr	81000		81000						
Cu	27000		34000	6000					
Ni	36000		---						
Zn	122000		150000	63000					
TBT									
Naphthalene	8		160	---	340				
Phenanthrene	32		240	11	1700				
Anthracene	5		85	---	290				
DBT	---		190	---	---				
Fluoranthene	39		600	12.2	110				
Pyrene	24		665	9	100				
Benz[a]anthracene	16		261	2.5	80				
Chrysene (Triphenylene)	20		384	8.1	---				
Benzo[a]pyrene	30		430	1.4	600				
Benzo[ghi]perylene	80		85	2.5	110				
Indeno[1,2,3-cd]pyrene	103		240	2.4	---				
CB28	0.22	1.7		0.75	3.2		0.1	64	
CB52	0.12	2.7		0.75	5.4		0.08	108	
CB101	0.14	3		0.7	6		0.08	120	
CB105	---	---		0.75	---		0.08		
CB118	0.17	0.6		0.6	1.2		0.1	24	
CB138	0.15	7.9		0.6	15.8		0.09	316	
CB153	0.19	40		0.6	80		0.1	1600	
CB156	---	---		0.6	---		0.08		
CB180	0.1	12		0.6	24		0.11	480	
γ-HCH			3	0.97	1.45			11	
α-HCH			---	0.64	---				
DDE (p,p')			2.2	0.63	---		0.1		
Hexachlorobenzene			20	0.63	---		0.09		
Dieldrin				---	---				

* Fish EAC passive for PCBs in lipid weight

Compiled Biological effects assessment criteria as used in this report are detailed in Table A2.2 below.

Table A2.2: Assessment criteria to be applied to biological effects data. Values for background assessment levels (BAC) and environmental assessment criteria (EAC) where relevant are given.

Biological Effect	Applicable to:	BAC	EAC
Vtg in plasma; µg ml^{-1}	Cod	0.23	
	Flounder	0.13	
EROD; pmol mg $protein^{-1}$ Pmol.min $^{-1}$mg protein $^{-1}$ S9 * pmol.min $^{-1}$ mg microsomal $protein^{-1}$	Dab (F)	178	
	Dab (M)	147	
	Dab (M/F)	680*	
	Flounder (M)	24	
	Plaice (M)	9.5	
	Cod (M/F)	145*	
	Plaice (M/F)	255*	
	Four spotted megrim (M/F)	13*	
	Dragonet (M/F)	202*	
	Red mullet (M)	208	
PAHs Bile metabolites; (1) ng ml-1; HPLC-F (2) pyrene-type µg ml^{-1}; synchronous scan fluorescence 341/383 nm (3) ng g^{-1} GC/MS * 1-OH pyrene ** 1-OH phenanthrne	Dab	16 (1) * 3.7 (1) ** 0.15 (2)	22(2)
	Cod	21 (1) * 2.7 (1) ** 1.1 (2)	483 (3) * 528 (3) ** 35 (2)
	Flounder	16 (1) * 3.7 (1) ** 1.3 (2)	29(2)
	Haddock	13 (1) * 0.8 (1) ** 1.9 (2)	35(2)
DR-LUC; ng TEQ kg^{-1}	Sediment	10	40
Bioassays; % mortality	Sediment, Corophium	30	60
	Sediment, Arenicola	10	50
	Water, copepod	10	50
Lysosomal stability (min)	Neutral Red Retention: all species	120	50
Comet Assay; % DNA Tail	*Mytilus edulis*	10	
Stress on Stress; days	*Mytilus* sp.	10	5
AChE activity; nmol.min^{-1} mg $prot^{-1}$ [1] gills [2] muscle tissue [3] brain tissue * French Atlantic waters ** Portuguese Atlantic waters [+] French Mediterranean Waters [++] Spanish Mediterranean Waters	*Mytilus edulis*	30 [1*]	21 [1*]
		26 [1**]	19 [1**]
	Mytilus galloprovincialis	29[1+]	20[1+]
		15 [1++]	10 [1++]
	Flounder	235 [2*]	165 [2*]
	Dab	150 [2*]	105 [2*]
	Red mullet	155 [2+] 75 [3++]	109 [2+] 52 [3++]
Liver histopathology;	Dab	Not applied	2

Biological Effect	Applicable to:	BAC	EAC
Macroscopic Liver neoplasms	**Dab**	**Not applied**	**2**
Scope for growth Joules hr $^{-1}$g dry wt.$^{-1}$	**Mussel (*Mytilus sp.*) (provisional, further validation required)**	**15**	**5**
Hepatic metallothionein µg g^{-1} (w.w.) **[1]Whole animal** **[2]Digestive gland** **[3]Gills** *** Differential pulse polarography** **** Sulphydryl method**	*Mytilus edulis*	**0.5 [1*]** **0.04 [1**]** **1.8 [2*]** **0.17 [2**]** **0.4 [3*]**	
	Mytilus galloprovincialis	**0.9 [1*]** **1.03 [1**]**	
		3.3 [2*] **0.3 [2**]**	
		0.6 [3*] **40 [3**]**	
Fish Disease Index	**Dab, flounder, cod, whiting, haddock**	**2.5% quantile**	**97.5% quantile**

Full details of the assessment criteria derivation and restrictions in use in ICES/OSPAR, 2010 and ICES/OSPAR, 2011 and Workshop on Assessment Criteria for Biological Effects Measurements (WKIMC ICES, 2009) reports at www.ices.dk and in the OSPAR background documents for individual biological effects methods.

Table A2.3: Assessment criteria and interpretations of the assessment classes for *Nucella lapillus* (OSPAR, 2004)

Assessment class	*Nucella* VDSI	Effects and impacts
A <BAC	VDSI = <0.3	Imposex (and hence TBT) close to zero (0 - ~30% of females have imposex)
B >BAC <EAC	VDSI = 0.3 - <2.0	Imposex below the EAC derived for TBT (~30 - ~100 % of the females have imposex)
C >EAC	VDSI = 2.0 - <4.0	Imposex higher than the EAC derived for TBT with a risk of adverse effects, such as reduced growth and recruitment
D >EAC	VDSI = 4.0 - 5.0	The reproductive capacity affected some females sterile.
E >EAC	VDSI = > 5.0	Populations unable to reproduce; majority, if not all females sterile
F >EAC	VDSI = -	Gastropod populations absent/expired

Table A2.4: EDC related assessment criteria and confidence estimates.

Compound/ criteria	E1	E2	EE2	NP	OP	ALP	Vtg	EEQ	Transition criteria	Colour transition	Comments
POCIS (ng device^{-1})	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	Presence/ Absence		Denotes presence/ absence only, not quantitative
AA EQS other surface waters WFD (ng L^{-1})	n/a	0.08^	0.007^	0.3 µg L^{-1}	0.012 µg L^{-1}	n/a	n/a	n/a	Pass/Fail		Pass/Fail clear transition. Total water basis
MAC EQS other surface waters WFD ng L^{-1} (µg L^{-1})	n/a	not derived	not derived	2 µg L^{-1}	0.13 µg L^{-1}	n/a	n/a	n/a	Pass/Fail		Pass/Fail clear transition. Total water basis
Relative potency to E2 by YES (converted concentration)	0.5 (0.16 ng L^{-1})1	n/a	n/a	0.000142	0.007 - 0.0011	n/a	n/a	n/a	Pass/Fail		Pass/Fail clear transition. EQS total water basis multiplied by relative potency.
WFD QS Biota used in assessment -limit to prevent secondary poisoning of predators	n/a	0.7 ng g^{-1}	0.07 ng g^{-1}	10 µg g^{-1}	10 µg g^{-1}	n/a	n/a	n/a	Pass/Fail		Pass/Fail clear transition.
WFD QS Biota used in assessment -human health via consumption of fishery products	n/a	3 ng g^{-1}	0.06 ng g^{-1}	8.7 µg g^{-1}	8.7 µg g^{-1}	n/a	n/a	n/a	Pass/Fail		Pass/Fail clear transition.
Vtg µg ml^{-1} plasma^^ BAC	n/a	n/a	n/a	n/a	n/a	n/a	0.13	n/a	Pass/Fail		Pass/Fail clear transition. BAC for flounder species specific
BCF mussels	not defined	not defined	not defined	2000-3000	not defined	n/a	n/a	n/a	BCF> 100 to trigger derivation of a quality standard referring to the protection of top predators from secondary poisoning		
BCF fish	51	6.5	610	1280	634	n/a	n/a	n/a			
EEQ sediment data: non-impacted Irish sites	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.32*	Represents average values from non-impacted Irish sites * EEQ in ng g^{-1} for sediment		
ALP/EEQ biota data: non-impacted Irish sites	n/a	n/a	n/a	n/a	n/a	11.46**	n/a	0.24***	**µg ALP mg protein $^{-1}$ *** EEQ in ng g^{-1} dw in biota		

[1]Routledge and Sumpter, 1997,

[2]Routledge and Sumpter, 1996,

^Proposed EQS only, E2 and EE2 are yet to be included in the WFD list of priority pollutants.

^^ Defined for flounder: UK provisional BC of 0.13 µg ml^{-1} based upon the 90th percentile of the entire male Vtg concentrations (range = <0.01–0.17 µg ml^{-1}, n = 95). Based upon the 90th percentile of male caged North Sea cod a provisional BC of 0.22 µg ml^{-1} is proposed for cod, Vtg concentrations range <0.01–1.35 µg ml^{-1} (n = 69).

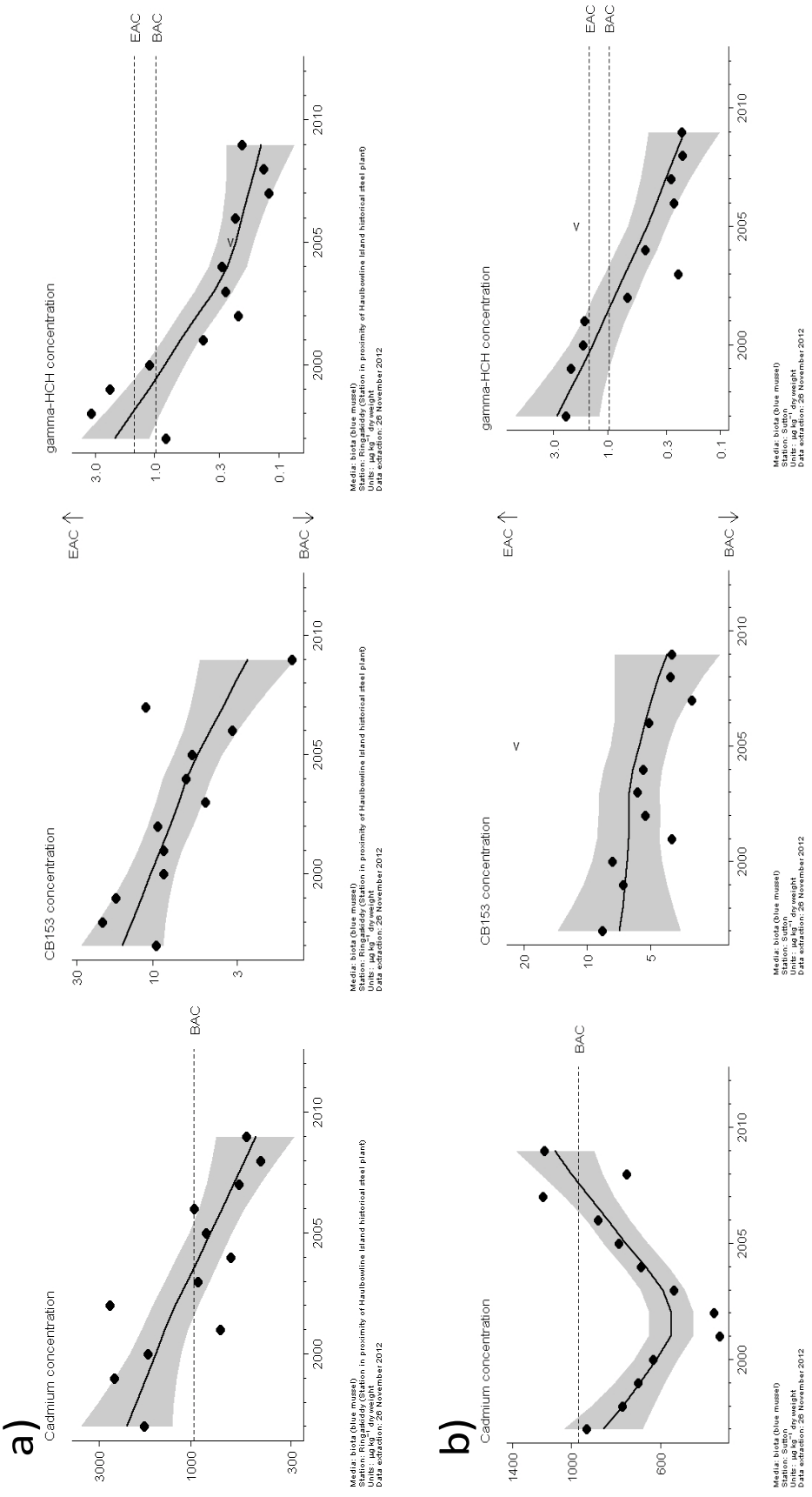
Table A2.5: Concentrations of contaminants in *Mytilus edulis* where no accepted assessment criteria are available (µg kg^{-1} dry weight) at Tier 1 locations around the Irish coast.

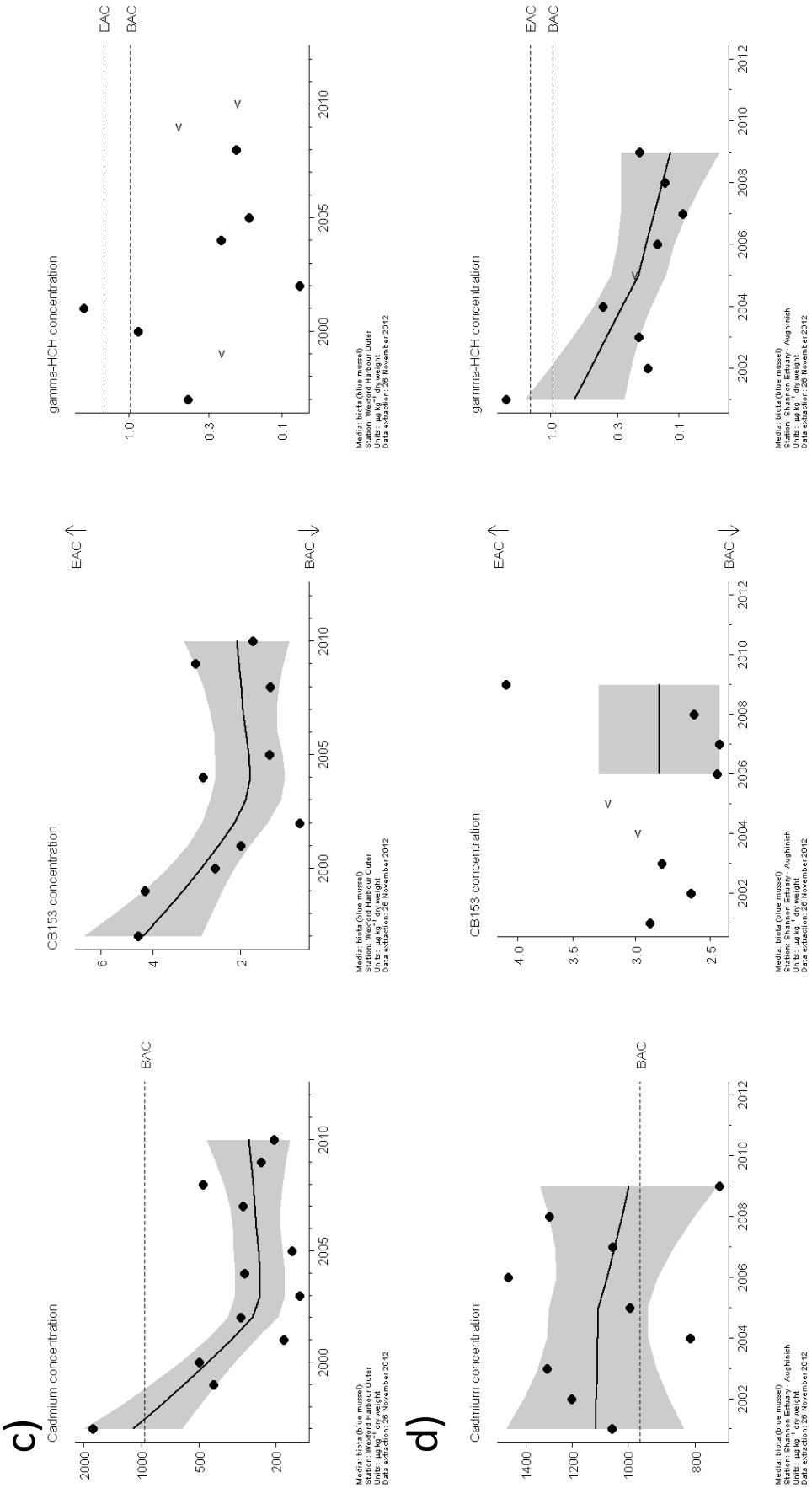
Compound	Shannon	New Quay	Tolka	Tralee	Cork	Kinvarra	Bantry	Wexford
2.4´-DDD	0.13	0.33	0.02	0.07	0.04	0.04	0.21	< 0.01
2.4´-DDE	NA	NA	NA	NA	0.006	NA	NA	< 0.01
2.4´-DDT	<0.02	(ND) 0.02	0.63	(ND) 0.13	(ND) 0.05	(ND) 0.05	(ND) 0.07	< 0.01
4.4´-DDD	NA	0.48	3.10	0.38	<3.07	0.39	6.66	< 0.01
4.4´-DDT	(ND) 0.01	(ND) 0.01	(ND) 0.03	NA	(ND) 0.01	(ND) 0.01	(ND) 0.01	< 0.01
Aldrin	(ND)0.02	(ND) 0.02	<0.38	(ND) 0.01	(ND) 0.01	(ND) 0.01	(ND) 0.01	< 0.05
α– Chlordane	NA	NA	NA	NA	0.006	NA	NA	< 0.01
α Endosulphane	NA	NA	NA	NA	0.006	NA	NA	< 0.05
β-Endosulphane	NA	NA	NA	NA	0.006	NA	NA	< 0.05
β -HCH	(ND) 0.01	0.19	0.57	<0.07	0.030	0.09	<2.96	< 0.01
δ-HCH	NA	NA	NA	NA	0.006	NA	NA	< 0.01
Dieldrin	0.120	0.19	3.070	0.52	1.110	0.68	2.27	0.06
EDS	0.100	0.03	NA	(ND) 0.01	(ND) 0.01	(ND) 0.01	(ND) 0.01	< 0.05
Endrin	(ND) 0.01	(ND) 0.02	0.110	(ND) 0.01	(ND) 0.01	(ND) 0.06	(ND) 0.01	< 0.01
γ-Chlordane	NA	NA	NA	NA	0.006	NA	NA	< 0.01
Heptachlor	0.030	0.10	0.07	<0.07	0.09	0.04	0.11	< 0.05
Mirex	NA	NA	NA	NA	0.006	NA	NA	< 0.01
Octachlorstyrol	NA	NA	NA	NA	0.006	NA	NA	< 0.01
Oxychlordane	<0.06	0.05	0.07	0.05	0.03	0.04	(ND) 0.01	< 0.01
T-chlordane	0.09	0.03	<1.36	0.28	0.23	0.20	1.04	NA
C-chlordane	0.15	0.12	<1.75	0.14	NA	0.12	0.51	NA
PCB	NA	NA	NA	NA	0.006	NA	NA	< 0.01
T-Nonachlor	0.12	0.17	0.32	0.16	0.14	0.11	0.32	< 0.01
BFR28	<0.09	< 0.04	1.02	(ND) 0.01	3.63	0.14	2.63	NA
BFR47	0.46	0.40	22.7	1.94	4.32	1.93	3.76	NA
BFR100	0.12	NA	4.65	1.01	1.09	0.42	0.35	NA
BFR99	0.14	0.08	<2.08	1.83	2.96	1.72	3.17	NA
BFR154	0.02	<0.06	< 0.12	(ND) 0.13	0.31	0.13	0.19	NA
BFR153	(ND) 0.01	<0.19	< 0.12	(ND) 0.01	0.60	0.42	0.90	NA
BFR183	(ND)0.02	<0.19	NA	<0.19	0.28	(ND) 0.03	(ND) 0.04	NA
Extractable Lipid	1.52	1.58	1.72	1.22	1.34	1.29	1.29	0.88

EDS=endosulphanesulphate

Appendix 3: Temporal trends used in assessment

Examples of supporting temporal trend plots for mussels from each of the tier II locations: a) Cork (Ringaskiddy), b) Dublin (Sutton), c) Wexford (Wexford Harbour Outer) and d) Shannon (Aughinish).




gamma-HCH concentration
EAC
BAC
CB153 concentration
EAC
BAC
gamma-HCH concentration
EAC
BAC
c)
Cadmium concentration
BAC
d)
CB153 concentration
EAC
BAC
Cadmium concentration
BAC

Appendix 4: Table of pressures and historical data at Tier I locations

Table A4.1: Summary details of sampling locations and associated pressures.

Location	GPS (general)	Potential pressures	Selected References
Dublin Bay (Tolka estuary, North Bank Lighthouse)	53° 21' 27.58 N 6° 11' 30.31 W (Number of sites sampled)	River Liffey; River Tolka; Industry; Shipping; Eutrophication. Sewage discharge: Ringsend WWTP (1, 640,000 PE). Nutrients: DIN and Phosphate Hydrocarbons, Metals, OTs, PCBs, PAHs,	Wilson, (2003), O 'Donnell et al. (2008), Buggy and Tobin, (2006) a and Choiseul et al. (1998), McBreen and Wilson, (2005), Tarrant et al. (2005), Minchin personal comm., Giltrap et al. (2009), Giltrap et al. (2008, 2012)
Cork harbour	51° 49' 53.46 N 8° 18' 02.82 W (Number of sites sampled)	Industry (pharmaceutical); Haulbowline site; Shipping Ringaskiddy: Historic steel plant. Sewage discharge: WWTP 413,000 plant PE, 323,000 agglomeration PE (Secondary treatment). Nutrients, Hydrocarbons, Metals, OTs, PCBs, PAHs,	Kilemade et al. (2004), Kilemade et al. (2009), Minchin et al. (1996)
Tralee Bay	52° 16' 36.75 N 9° 49' 29.62 W	Shipping; Sewage discharge, Metals (cd)	Minchin et al. (2003), Falvey J.P.H University of Dublin Thesis (1995)
Kinvarra Bay	N56.14097 W008.93758	Small harbour; Raw sewage discharge PE 850. Subject to input of groundwater from large freshwater catchment	Cave and Henry (2011) and Wilson and Rocha (2012)
Wexford harbour	52° 20'22.67 N 6° 27'18.37 W (Number of sites sampled)	Eutrophication; Sewage discharge Shipping; Pig farm; Landfill run off. Sewage discharge: 45,000 PE (30,000= plant PE, 17,000=agglomeration PE**) Secondary treatment with NR	Costello et al. (2000) (EPA report)
Bantry Bay	51°40' 50.54 N 9° 27' 16.48 W	Aquaculture in vicinity Metals, OTs, HCs	Enterprise Ireland (unpublished data)
Galway Bay	53° 14' 27.60 N 9° 44' 19.19 W	Sewage discharge: Mutton Island WWTP (91,600PE) . Secondary treatment, agricultural run-off from river Corrib and others, inputs of groundwater	
Newquay	53° 09'27.28 N 9° 04' 03.84 W	Little anthropogenic input, possible agricultural run-off and input of untreated waste from domestic sources	Giltrap (2008), Giltrap et al. (2009, 2011), Glynn et al. (2001)
Shannon (Carraigaholt)	52° 37.670 N 009° 19.930' W	River Shannon drains a large catchment with urban, agricultural and industrial influences. Carrigaholt (PS) and outer Shannon Estuary (fish) main sampling area.	O' Leary and Breen (1998)
Omey Island	53° 31.8 N 10° 10. 0 W	Few anthropogenic pressures. Limited agricultural run-off and input of untreated waste from limited domestic sources.	Giltrap PhD Thesis (2008)

WWTP data from EPA Urban Waste Water Discharges in Ireland: A Report for the Years 2006 and 2007, Monaghan et al. 2009 and Environmental Protection Agency, Ireland: www.epa.ie.

Appendix 5: Project deliverables and milestones (Summary information)

Deliverables	Milestones	Complete	Comment
a) Establishment of LMS technique and screening of biomarker	Time of year for assay	Yes	LMS not used in TIER 1 due to time of year difference with SFG
b) Metallothionein (MT) measurement in fish/mussels	Development of assay	Partial	Method up and running but minor alterations required Method in fish is no longer recommended as part of integrated approach
c) EROD establishment of method	Development of assay	Partial	Time constraints and staff issues did not permit optimization of method. Samples were subcontracted
d) EROD analysis of samples	3 month degradation period exceeded in liver samples.	Yes	Time and staff limitations for method development at MI. Samples analysed from 6 tier 1 locations
e) PAH metabolites in fish	Development of method	No	Determined that method setup not cost efficient for the number of samples to be analysed. Subcontracted
f) Scope for growth and condition factor	Development of method and analysis	Yes	Completed for TIER1 sites.
g) Fish Pathology and Fish Disease Index	Onboard and laboratory training	Yes	Fish disease expertise up and running at MI
h) Sediment Toxicity	Other recommended analysis development including Arenicola marina and whole sediment tests	Yes	Additional tests were developed by SATL and additional samples were analysed with the battery of bioassays which was outside the scope of the project
i) Genetic assays: Comet assay and DNA strand breaks	Comet assay development and use for Tier II	Yes	COMET assay used in caging study. Staff changes at DIT resulted in use of the COMET assay for mussels only and no DNA strand break assay was set up.
Biological effects of endocrine disruption (WP3)			
j) Alkali labile phosphate	Method development and analysis	Yes	Complete for nine Tier 1 locations, 4 Tier II locations and caging studies
k) Vitellogenin	Method development and analysis	No	Samples subcontracted as there was no access to dab antibody which would have taken extra time and funding to develop at TCD
l) Imposex	Method development and analysis/ QUASIMEME performance analysis	Yes	All complete and method up and running at TCD
Other biological effects techniques developed outside of original project			
m) Stress on stress	Method development	Yes	Completed as part of Tier 1 analysis
n) Ferric Reducing Ability of Plasma (FRAP)		Yes	Oxidative stress assay up and running at TCD
o) Intersex in marine snails		Yes	Completed on same stations as imposex

Deliverables	Milestones	Complete	Comment
p) Mussel histopathology including gametogenesis		Yes	Completed on all samples to link with biomarkers for gonadal stage/ histopathology
Selection of TIER I and TIER II sampling and analysis (WP2)	Literature review with available data and steering meeting	Yes	Tier I sites chosen based on historical data. Tier II sites chosen based on Tier I data
Benthic sampling and analysis	Availability of research vessels (day grab equipment)	Yes	All Tier II samples taken at the same time of year with exception of Dublin Bay.
Chemical analysis (WP4)			
a) Literature review	None	Yes	Completed as part of Jenny Ronan PhD, 2013 and case study 2 in this report
b) Analysis of EDCs	Development of methodologies for range of EDCs and analysis Sampling and analysis	Yes Yes	Complete for E1, E2, EE2, NP and OP (see case study) Complete (EDCs in water and biota in-house methods developed (Ronan and McHugh, 2013). POCIS PS devices subcontracted.
c) Trace metals and chlorinated organics	Sampling and subcontracting	Yes	Completed due to resource issues. In-house capacity already developed
d) PAH/Hydrocarbons measurement in marine matrices	Sampling and analysis (method development)	Yes	Completed. PAH analysis in biota and sediment now completed in-house. PAH analysis capacity in PDMS passive samplers also completed.
e) Organotin analysis (OTs)	None	None	None completed within project. Imposex used as more sensitive measure of TBT pollution
f) Passive sampling	Development of PS methodologies	Yes	PDMS hydrophobic analysis (PAH and PCB) completed in linked PhD project. Development of POCIS sampling procedures completed as part of Jenny Ronan PhD. Future POCIS will be subcontracted also.
d) Stable Isotopes (SI) and Trophic level assessment	None		Not a core technique. High sampling and resource input required. It was decided by an advisory group not to use this techniques as part of this project and to concentrate time on core methods
Data Integration and Interpretation of Results (WP5)	Collation of all data	Yes	Complete as integrated assessment framework in this report

Appendix 6 Summary of Benthic indices per site and per sample

Table 5.6 Summary of Benthic Indices per site and sample (site-sample) from A) Wexford; B) Dublin and C) Cork, showing number of species (S), number of individuals (N), Simpson's diversity (d), Pielou's Evenness (J), Rarefaction-derived number of species per 100 individuals sampled (ES(100)), Shannon-Weiner Index (H', $\log_2$), and Simpson's λ (as 1-lambda)

3A	Wexford						
	S	N	d	J'	ES(100)	H'(log2)	1-lambda'
1-1	49	273	8.56	0.68	28.82	3.83	0.84
1-2	46	189	8.58	0.73	32.67	4.04	0.85
1-3	34	235	6.04	0.63	22.38	3.19	0.75
1-4	33	363	5.43	0.51	17.26	2.57	0.66
1-5	28	144	5.43	0.72	22.67	3.47	0.84
2-1	2	2	1.44	1.00	2.00	1.00	1.00
2-2	6	7	2.57	0.98	6.00	2.52	0.95
2-3	30	85	6.53	0.86	30.00	4.20	0.93
2-4	6	6	2.79	1.00	6.00	2.58	1.00
2-5	2	2	1.44	1.00	2.00	1.00	1.00
3-1	10	17	3.18	0.96	10.00	3.18	0.93
3-2	6	8	2.40	0.97	6.00	2.50	0.93
3-3	12	27	3.34	0.81	12.00	2.92	0.82
3-4	26	45	6.57	0.93	26.00	4.38	0.96
3-5	38	183	7.10	0.70	26.26	3.66	0.86
4-1	14	18	4.50	0.98	14.00	3.73	0.97
4-2	18	47	4.42	0.91	18.00	3.79	0.93
4-3	17	39	4.37	0.86	17.00	3.51	0.89
4-4	17	35	4.50	0.83	17.00	3.38	0.87
4-5	37	263	6.46	0.70	22.91	3.64	0.86
3B	Dublin						
1-1	16	26	4.60	0.89	16.00	3.56	0.91
1-2	30	69	6.85	0.90	30.00	4.40	0.95
1-3	29	158	5.53	0.74	23.54	3.58	0.85
1-4	19	101	3.90	0.81	18.93	3.43	0.88
1-5	23	139	4.46	0.72	19.65	3.26	0.84
2-1	17	43	4.25	0.90	17.00	3.66	0.92
2-2	26	129	5.14	0.72	23.11	3.37	0.81
2-3	20	64	4.57	0.86	20.00	3.72	0.91
2-4	13	45	3.15	0.79	13.00	2.91	0.82
2-5	22	55	5.24	0.82	22.00	3.65	0.88
3-1	49	267	8.59	0.85	34.06	4.75	0.94
3-2	42	192	7.80	0.81	31.44	4.38	0.92
3-3	51	250	9.06	0.84	34.27	4.75	0.95
3-4	54	243	9.65	0.82	34.60	4.72	0.94
3-5	53	231	9.55	0.87	37.65	5.00	0.96
4-1	61	1413	8.27	0.37	15.41	2.18	0.51
4-2	62	393	10.21	0.79	33.69	4.71	0.93
4-3	61	509	9.63	0.72	28.78	4.27	0.90
4-4	49	262	8.62	0.77	30.54	4.31	0.91
4-5	62	399	10.19	0.76	32.41	4.53	0.92
5-1	15	25	4.35	0.94	15.00	3.68	0.95

5-2	29	101	6.07	0.74	28.84	3.57	0.82

	S	N	d	J'	ES(100)	H'(log2)	1-lambda'
5-3	20	32	5.48	0.92	20.00	3.99	0.95
5-4	24	87	5.15	0.65	24.00	2.97	0.71
5-5	42	200	7.74	0.55	27.85	2.99	0.64
3C	**Cork**						
1-1	52	352	8.70	0.66	28.22	3.74	0.80
1-2	53	163	10.21	0.84	40.32	4.82	0.94
1-3	51	176	9.67	0.81	36.95	4.57	0.92
1-4	49	162	9.43	0.84	37.88	4.72	0.94
1-5	39	133	7.77	0.81	33.37	4.29	0.92
2-1	24	73	5.36	0.76	24.00	3.50	0.86
2-2	42	534	6.53	0.40	17.43	2.15	0.49
2-3	33	180	6.16	0.47	22.53	2.36	0.53
2-4	22	110	4.47	0.57	20.70	2.52	0.66
2-5	Missing						

Appendix 7 Supporting case studies

With student moderatorship, MSc. projects and the help of research technicians, it was possible to provide extra baseline data for the Sea Change project. Some of these supporting case studies are described below.

i. In vivo screening of EDCs in M. edulis
Ronan et al., 2013 (in preparation)

Endocrine disruption in the marine environment is a major concern. Knowledge of endocrine disruption in mussels is still very limited. In order to understand exposure and effects in the field, it is imperative to first understand the specific effects of these EDCs in the laboratory. This study aimed to investigate *in-vivo* exposure of a model estrogenic compound 17α ethynylestradiol (EE2) on the marine mussel *Mytilus edulis* under a submerged and intertidal regime. A 7 day exposure was performed at the SATL where effects of this compound were investigated with the ALP method which detected levels of vitellin like protein in the mussel gonad. Tissue and water concentrations of EE2 were also measured using developed methodology. A significant induction of ALP was determined after 7 days indicating endocrine disruption in these mussels. Tissue concentrations were higher in intertidal mussels than submerged suggesting that intertidal mussels may be more susceptible to endocrine disruption. This forms an important basis and understanding of the effects of endocrine disruption. More research is warranted in future studies in order to perform similar exposures with varied levels that are determined in the field.

ii. Caging studies

Caging studies have been recommended for integrated monitoring and are recognized as a valuable biomonitoring tool as a range of confounding factors can be eliminated such as size, genetic shifts and sexual maturity (ICES, 2013). Transplanting studies have previously been used to provide valuable biological effects information (Giltrap et al., 2009).

Two major caging studies took place during the course of the Sea Change project. The first being a pilot mussel transplantation study at the North Bank Lighthouse (NBL) in Dublin Bay where mussels were sampled three times over a period of 6 months and analysed for contaminants and biological effects. There was a significant induction of ALP between the initial time (August) and first sampling time (October). There was a significant difference between ALP values at each sampling event at the control site indicating a potential seasonal cycle in ALP levels and highlighting the requirement for seasonal studies with this biomarker. Differences were also noted for the MT at the control site thus seasonal studies are warranted in relation to biotic variation of this biomarker.

Following on from this pilot exercise, a more in depth caging study was performed in the summer of 2010 at the three locations Omey Island (reference site), Mutton Island and Dublin Bay (NBL). Testing included a range of biomarkers including MT, AChE, ALP and COMET assays and chemical testing of a range of EDC compounds. Overall, effects were induced at Mutton and Omey Island with the COMET assay and MT and effects on the Dublin transplants were lower (Rochford, 2012). The combined multi-compartment, multi-parameter approach in this study enabled the presence of estrogenic EDCs, and EDC associated effects to be assessed at three Irish coastal sites. Caging studies were determined to be an extremely useful tool in integrated monitoring and are recommended for future integrated monitoring where resident species may be absent.

iii. Intersex in marine snails
Sarah Ebrill moderatorship project 2012

The intersex phenomenon in *Littorina littorea* is a known alternative to imposex in *N. lapillus* albeit less sensitive however is useful to collect and analyse samples where *N. lapillus* populations are absent. During the imposex survey, *L. littorea* were sampled from 35 coastal sites in 7 geographic locations around Ireland and were later analysed for TBT induced intersex, stages 0 – 4. An intersex index (ISI), an average of the stages of intersex, was calculated for each site. Overall highest ISI values were determined in Wexford including two Kilmore Quay sites and one in Wexford Harbour in close proximity to the Tier I location. A correlation was investigated between VDSI and ISI however no significant correlation could be established between these indices, possibly due to the low ISI values and sensitivity of *L. littorea*. This study confirms that *L. littorea* is a suitable organism for sampling where *Nucella* are absent.

iv. Parasite effects on biological effect measurements
Miriam Whittle, 2012 and Sean Kelly, 2011

Infection by parasites has been shown to have negative effects on the health of aquatic populations (Baudrimont et al. 2006). Pollution has the effect of increasing parasite burden by increasing host vulnerability (Lafferty and Kuris, 1999). Parasite burdens have been shown to affect biomarker response (Bignell, personal communication). This hypothesis was investigated with two moderatorship projects during the course of the Sea Change project. In one project, scope for growth was investigated along with parasite prevalence in mussels sampled from the Tier I locations, Tolka estuary and Wexford Harbour. In general two species were encountered; the trematode *Renicola roscovita* and copepod *Mytilicola intestinalis*. It was concluded that presence/absence of parasitic infection had no significant impact on the SFG response. The second project focused on the link between parasite prevalence and the biological effect stress on stress and the respiration element of SFG. Numbers of parasitized mussels were limited for SOS and no definitive conclusion could be made. It was reported that infected mussels had significantly higher respiration rates than uninfected mussels. This indicates the potential for parasitic infection to interact with the physiological response of the host and could have implications for the outcomes of ecotoxicological studies, with parasitism representing a potential confounding factor. Further studies are therefore warranted based on this data.

v. Age classification and growth rates in dab in relation to biochemical measurements

This was a study of the age classification and growth rates of dab at the four Tier II locations sampled around Ireland. The main aim was to assess whether growth rates and age differentiated between sites. The age of the fish is important when investigating biomarker studies due to the exposure time of the contaminants. Whole otoliths were extracted and read to estimate the age of individual fish. In analysis of the lengths at age classes it was found that there were no significant differences in fish of the same age between sites which suggest that dab sampled were relatively constant in size at age at the four different locations. Results of this study can be used as a good baseline for further work on Irish dab populations in relation to biomarker responses. In addition to this, a method for age determination was set up in TCD. Age measurements are far more accurate than size measurements as a proxy for age and therefore can be used in future monitoring programs.

vi. Gametogenic cycle relationship to the lysosomal stability measurement (LMS) in *M. edulis* Giltrap et al., 2013 (in preparation)

Initially, LMS was outlined as a primary biomarker as part of the Tier I screening. A requirement of this biomarker is that sampling is conducted at a particular time of year due to the variability in the response with season. This differs from the optimal time of year for sampling SFG, therefore it was not possible to use this biomarker in combination with SFG for Tier I screening. This study was performed in order to investigate the seasonal variability of this response in mussels taken from a control location. Mussel histopathology was monitored in mussels with a number of parameters including the reproductive status, inflammatory response, brown cell inflammation, atrophy of digestive gland tubules, kidney lipofuscin and the presence of parasites were investigated in combination with LMS. The effect of multiple parameters on the biomarker response was investigated using a generalized linear mixed model (GLMM) with R 2.14.2 software. There was a wide variation in the response of the biomarker seasonally (See Figure 5.4) with highest values in Feb-Apr indicating this as a good time of year for sampling and analysis. These results will further elucidate the cause of the variation in the biomarker response. It was determined that gonad stage and spawning in the individual mussels had a significant effect on the biomarker response ($Pr = < 0.05$).

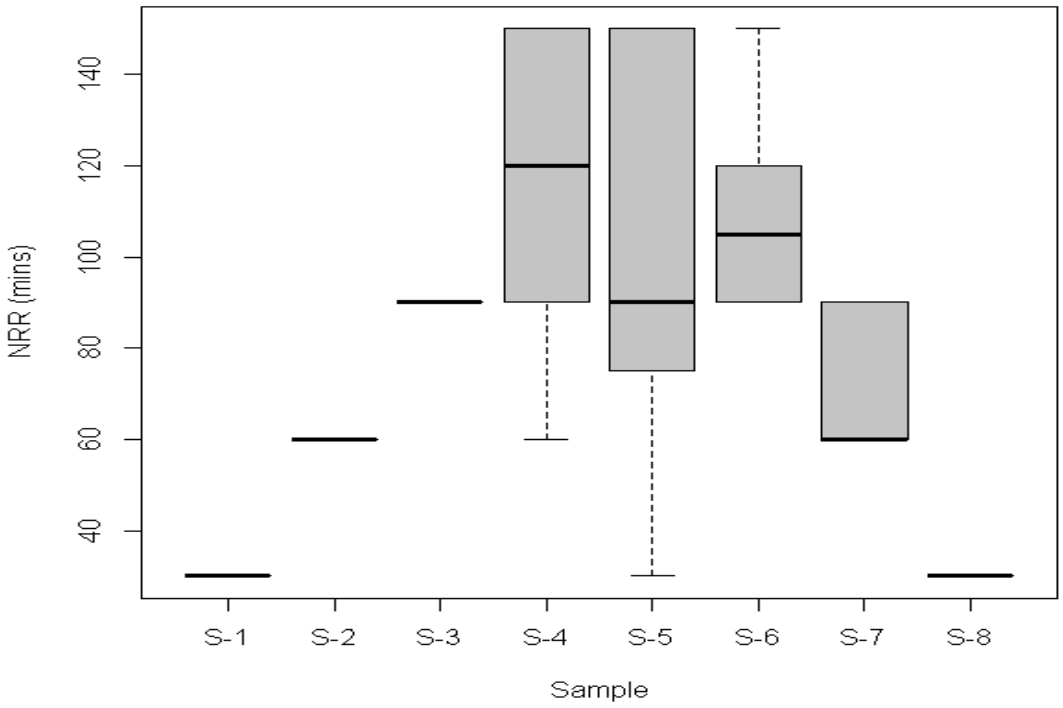
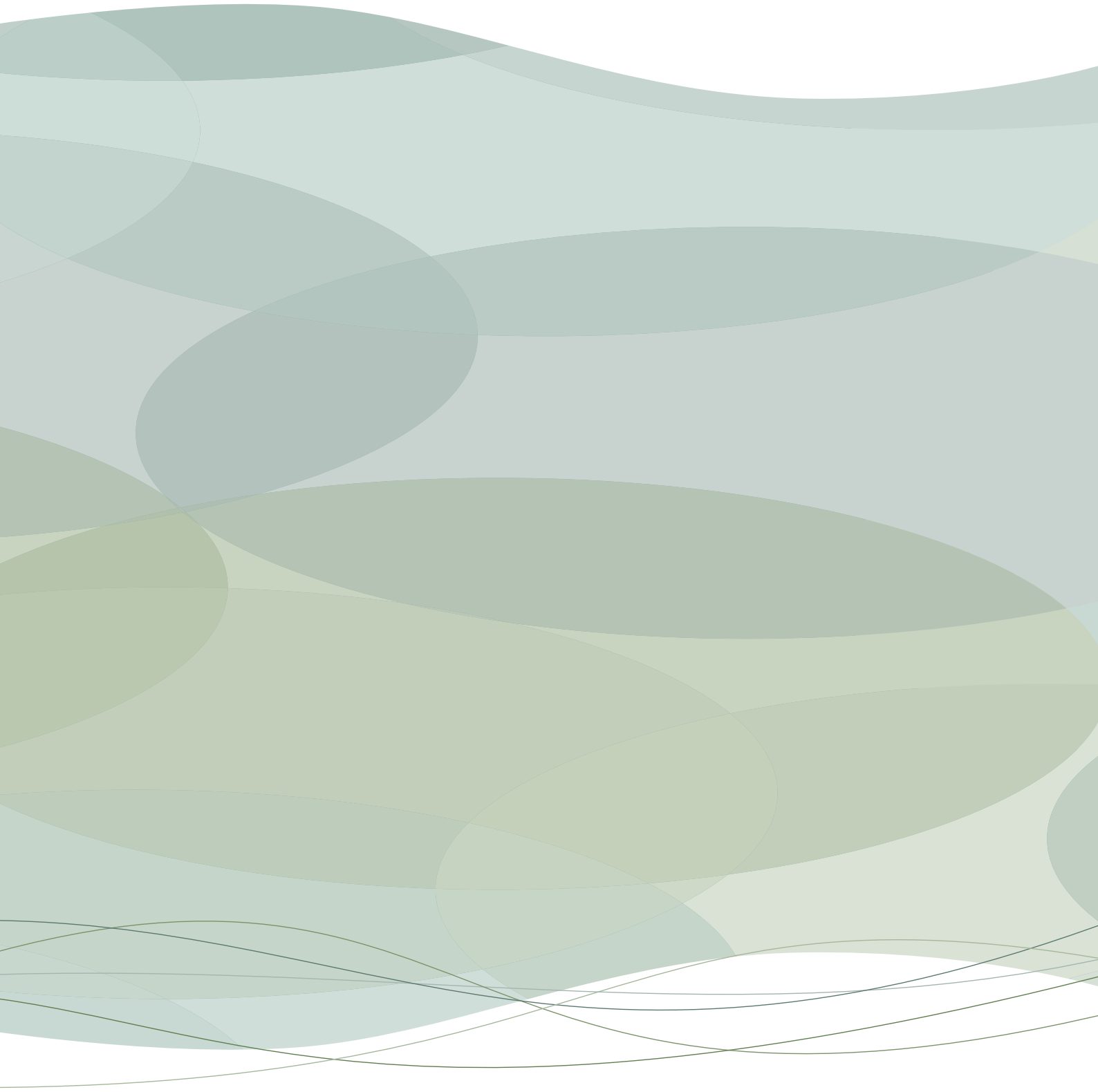


Figure 5.5: Lysosomal membrane stability (neutral red retention time) of haemocytes from mussels (*Mytilus edulis*) sampled from Aughinish Bay at various times over the period of one year. Data are shown as group median (+stdev) (n = 10) in each replicate group (S-1: Sep-09, S-2:Nov-09, S-3: Dec-09, S-4: Feb, S-5: Mar, S-6: Apr, S-7:May, S-8:Jul)

These studies further enhance our knowledge of seasonal and parasite (natural) effects on biomarker/population responses and are vital in terms of supporting response based measurements and in understanding biotic variation. Further studies such as these are essential for understanding and interpretation of biomarker data for future monitoring programs.



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