

PROJECT COLLABORATORS

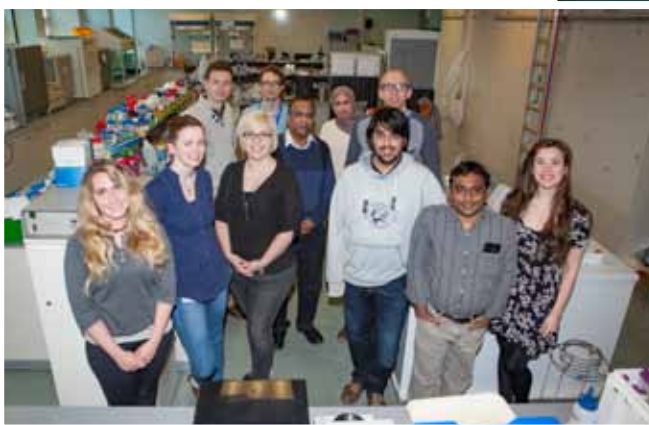
RESEARCH WITH PLYMOUTH UNIVERSITY



BIOLOGICAL &
MARINE SCIENCES
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MICROSCOPY
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“ Our work involves the development and validation of novel, reliable *in vitro* fish methods as an alternative to *in vivo* studies. ”

CONTACT DETAILS

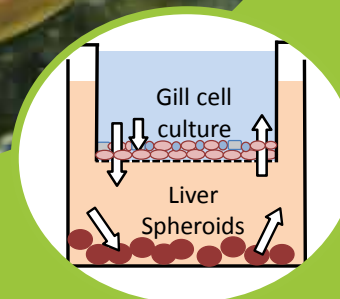
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Virtual Fish (2014-2017)

Moving up a dimension: 3D *in vitro* models
as effective alternatives to live fish studies

The
Virtual Fish Project
has received funding
from the BBSRC
and NERC
(grant number
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For more information,
please visit the project website:

 [https://www.plymouth.ac.uk/research/
genetic-ecotoxicology/virtual-fish](https://www.plymouth.ac.uk/research/genetic-ecotoxicology/virtual-fish)

INTRODUCTION

Whilst fundamental life processes are traditionally studied at whole organism level (*in vivo*), in recent years for economic, ethical and legal reasons, there has been much emphasis on the use of cells, tissues or organs which are grown outside the body in plastic dishes or flasks, the so called *in vitro* system. The use of such *in vitro* models in fish-based studies has the benefit of requiring only a few donor animals to provide a large number of cultures suitable for experimental Testing. This is in line with Reduction and Refinement of the 3Rs principles.

We have developed a specialised culture of fish liver 'spheroids' that mimic the responses of whole tissue. The development of a functional gill and gut primary culture would allow the aqueous and dietary uptake routes to be modelled *in vitro*. We hypothesise that these cultures could be combined with the liver spheroids to create a co-culture system where uptake and metabolism could be modelled in one experimental unit.



CHARACTERISATION AND BIOLOGICAL EFFECTS

Virtual Fish project aims to adopt an integrated approach to provide a mechanistic understanding of the biological responses of the gill, gut and liver culture types through seeding, establishment and maturation. Novel 3D cultures have been developed that are better able to mimic the responses observed *in vivo*, and have a longer viability than current models.

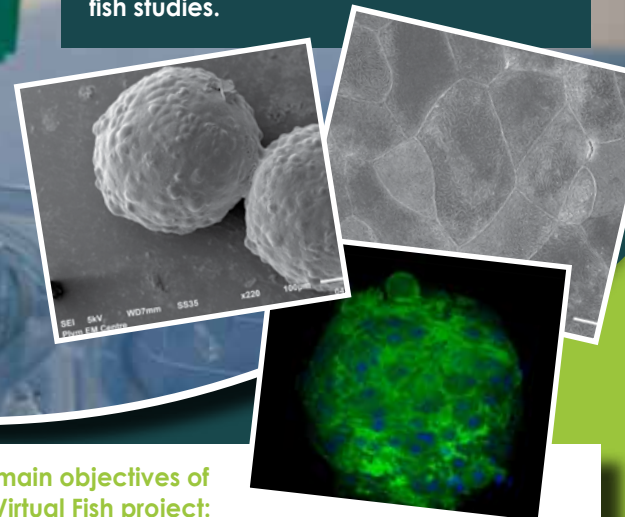
Thorough characterisation of these cultures through histological, physiological and biochemical endpoints will indicate their best use in future experiments. We also plan to expose cultures, in single and combination to common environmental contaminant (e. g. metal, PAH, pharmaceutical), at appropriate concentrations and record biomarker responses, metabolism and accumulation. The large data set generated will characterise each culture type and allow comparison to the parent organ processes and exposure responses *in vivo*. By continual refinement and development of culture techniques, more realistic *in vitro* models can be produced that better mimic the *in vivo* response and offer a genuine alternative to live fish studies.

CHEMICAL ANALYSIS

Thorough chemical analysis will be carried out using state-of-the-art analytical techniques. This will determine concentrations of exposure contaminants in both tissues and media and identify metabolism products. Quantification of metals is determined using ICP-MS to track accumulation in different compartments. PAHs (e.g. BaP) are quantified in tissues by ultrasound-assisted extraction and GC-MS. Pharmaceuticals are typically quantified by LC-MS/MS. Immunohistochemical staining and Electron Microscopy techniques are also employed.

OBJECTIVES

The project aims to probe the primary hypothesis that a co-culture of 3D *in vitro* cultures (liver-spheroids and gill/gut layer cultures) from rainbow trout (*Oncorhynchus mykiss*) can represent an effective alternative model to live fish studies.



The main objectives of the Virtual Fish project:

1. Characterise and define the culture conditions to ensure functionality and viability are prolonged.
2. Establish baseline values and compare key biomarkers between *in vitro* cultures and *in vivo* data, from both literature and in-house studies.
3. Determine the functionality of cultures in bio-transformation pathways and metabolite formation following exposures to reference contaminants (PAHs, Pharmaceuticals, Metals).
4. Compare the comparative physiology (oxygen uptake, bio-energetics, metabolism and excretion rates) of the systems in response to 'normal' control conditions and contaminant exposures.
5. Evaluate 'mixture' toxicity from a 'toxicogenomic' perspective in both isolated tissue preparations and co-cultures.
6. Measure the bioconcentration of selected chemicals in all tissue types following chronic exposures using cutting edge analytical techniques.
7. Communicate the methodologies and findings to key industrial, academic and stakeholder groups.

