RESEARCH WITH PLYMOUTH UNIVERSITY

BRUSSELS 2017
SETAC EUROPE

Primary culture of rainbow trout liver & gill

- 10th May 2017 SETAC Europe 27th Annual Meeting, Brussels

- Workshop:
- Current trends in fish *in vitro* toxicology: Applications of 3Rs principles

- Dr Richard Maunder, School of Biological and Marine Science, Plymouth University

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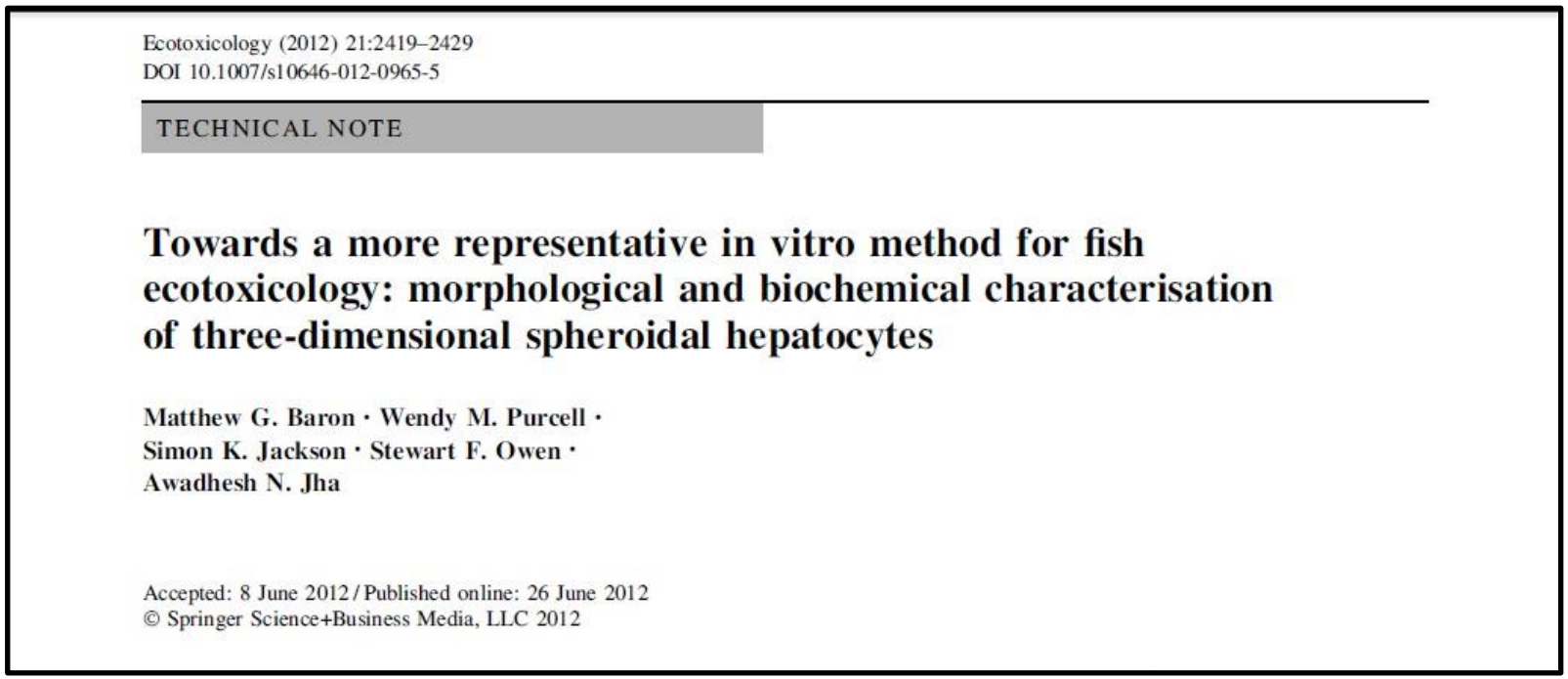
Liver spheroid production

Methods of Dr Matt Baron;

see Baron *et al. (*2012) Ecotoxicology 21, 2149-2429.



Ecotoxicology (2012) 21:2419–2429
DOI 10.1007/s10646-012-0965-5

TECHNICAL NOTE

Towards a more representative in vitro method for fish ecotoxicology: morphological and biochemical characterisation of three-dimensional spheroidal hepatocytes

Matthew G. Baron · Wendy M. Purcell · Simon K. Jackson · Stewart F. Owen · Awadhesh N. Jha

Accepted: 8 June 2012 / Published online: 26 June 2012

SoP is available on request

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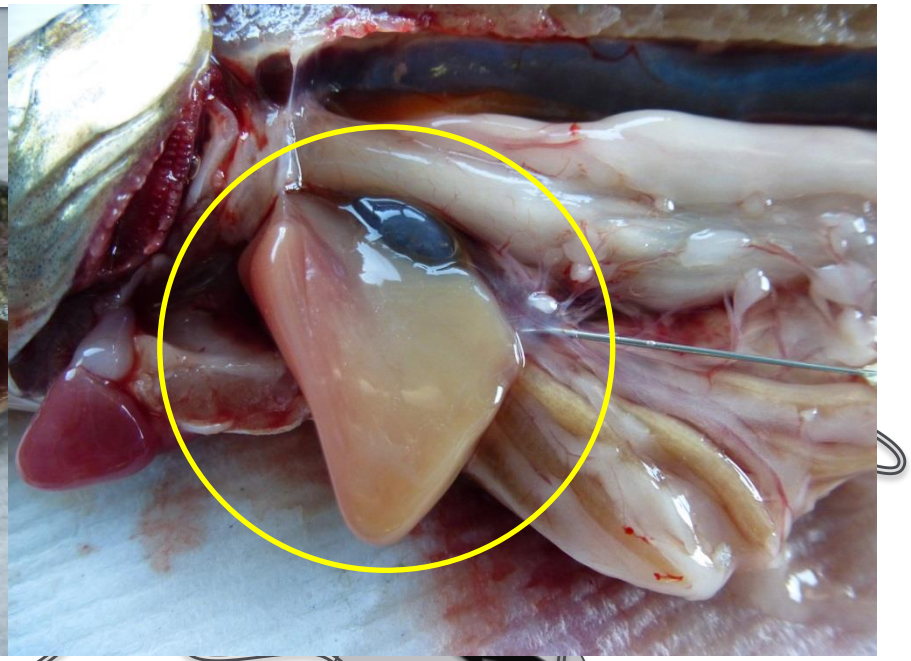
Liver spheroid production

In situ perfusion



HBSS blood clearance

Collagenase digestion

Culture media wash

Peristaltic pump

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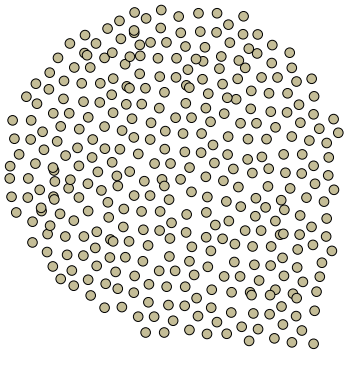
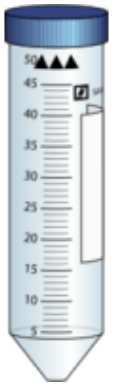
Liver spheroid production 2

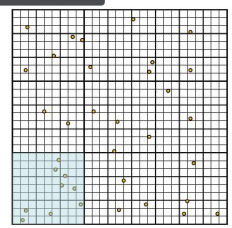
wash, count, plate

Wash hepatocytes to purify

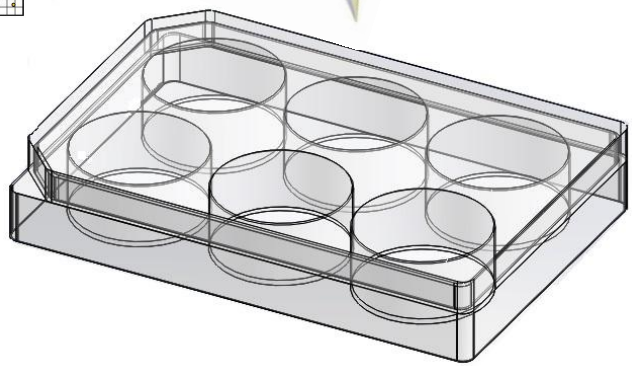




Haemocytometer count of cells



Seed at 1 million cells per mL



3 mL L-15 media +10% FBS per well (6 well plate pre-treated with Poly(2-hydroxyethylmethacrylate))

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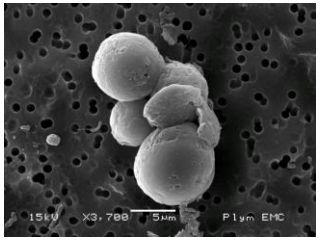
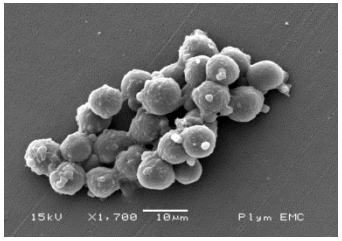
Liver spheroid production 3

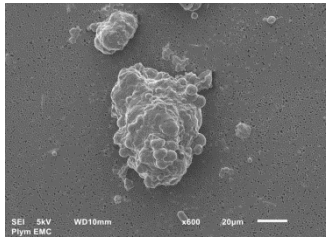
rotate, renew, clean

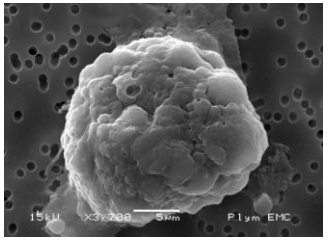
Clean culture and renew 1.5 mL media every other day

Day 1

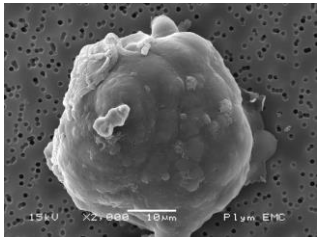








Day 7



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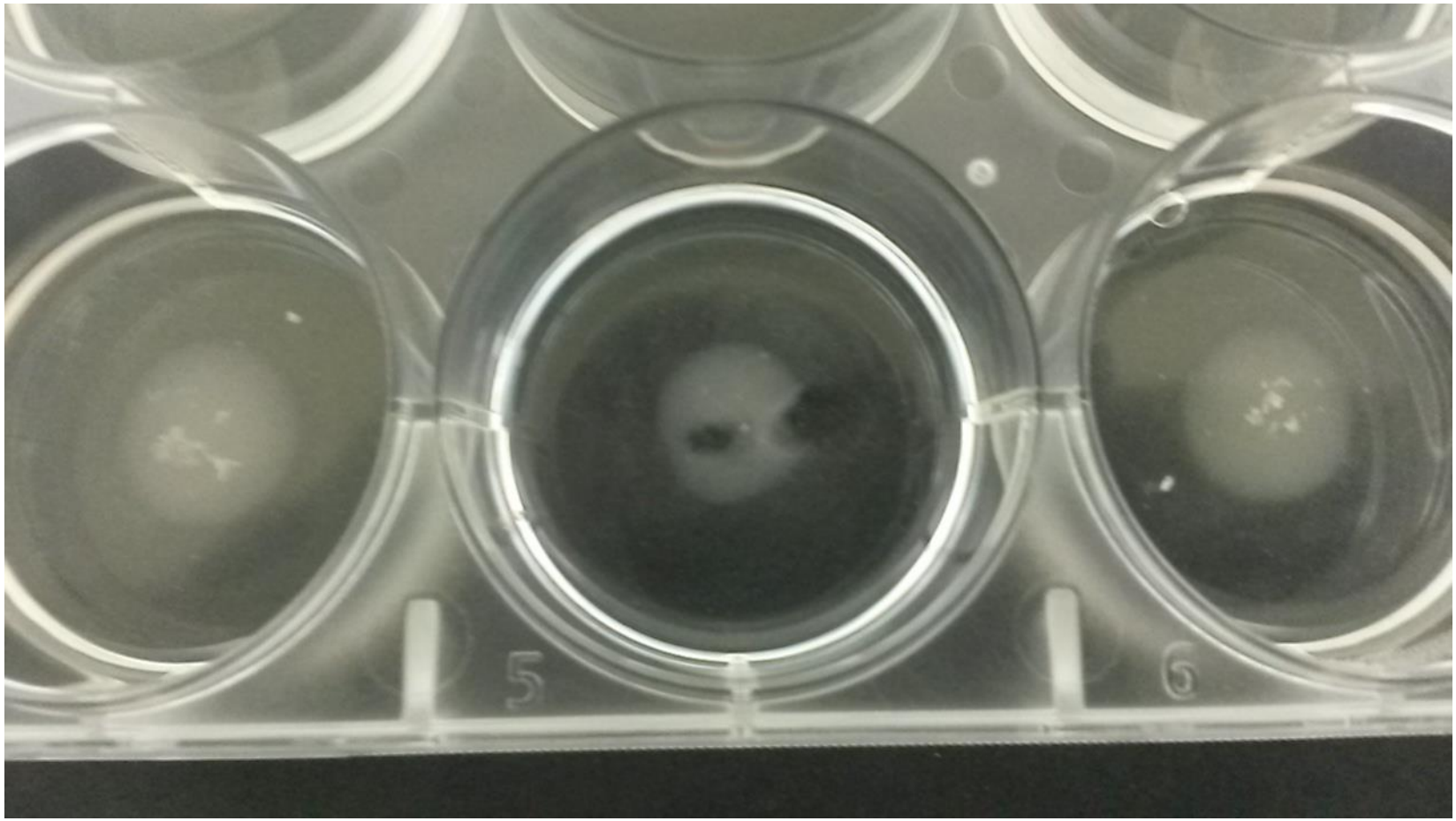
Liver spheroid production 3

rotate, renew, clean



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Spheroid metabolism assay

see Baron *et al. (*2017) PLoS ONE 12(1): e0168837

PLOS | ONE

RESEARCH ARTICLE

Pharmaceutical Metabolism in Fish: Using a 3-D Hepatic *In Vitro* Model to Assess Clearance

Matthew G. Baron[1,2], Kate S. Mintram[1,2], Stewart F. Owen[2]*, Malcolm J. Hetheridge[2], A. John Moody[1], Wendy M. Purcell[3], Simon K. Jackson[3], Awadhesh N. Jha[1]*

1 School of Biological Science, Plymouth University, Devon, United Kingdom, **2** AstraZeneca, Alderley Park, Macclesfield, Cheshire, United Kingdom, **3** School of Biomedical & Healthcare Science, Plymouth University, Devon, United Kingdom

* Stewart.Owen@AstraZeneca.com (SFO); a.jha@plymouth.ac.uk (ANJ)

Check for updates

Abstract

At high internal doses, pharmaceuticals have the potential for inducing biological/pharmacological effects in fish. One particular concern for the environment is their potential to bioaccu-

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Spheroid characterisation

see Langan *et al. (*2015) PLoS ONE 11(2): e0149492

Spheroids:
Metabolic activity > S9 fractions / hepatocytes
Viable for several weeks

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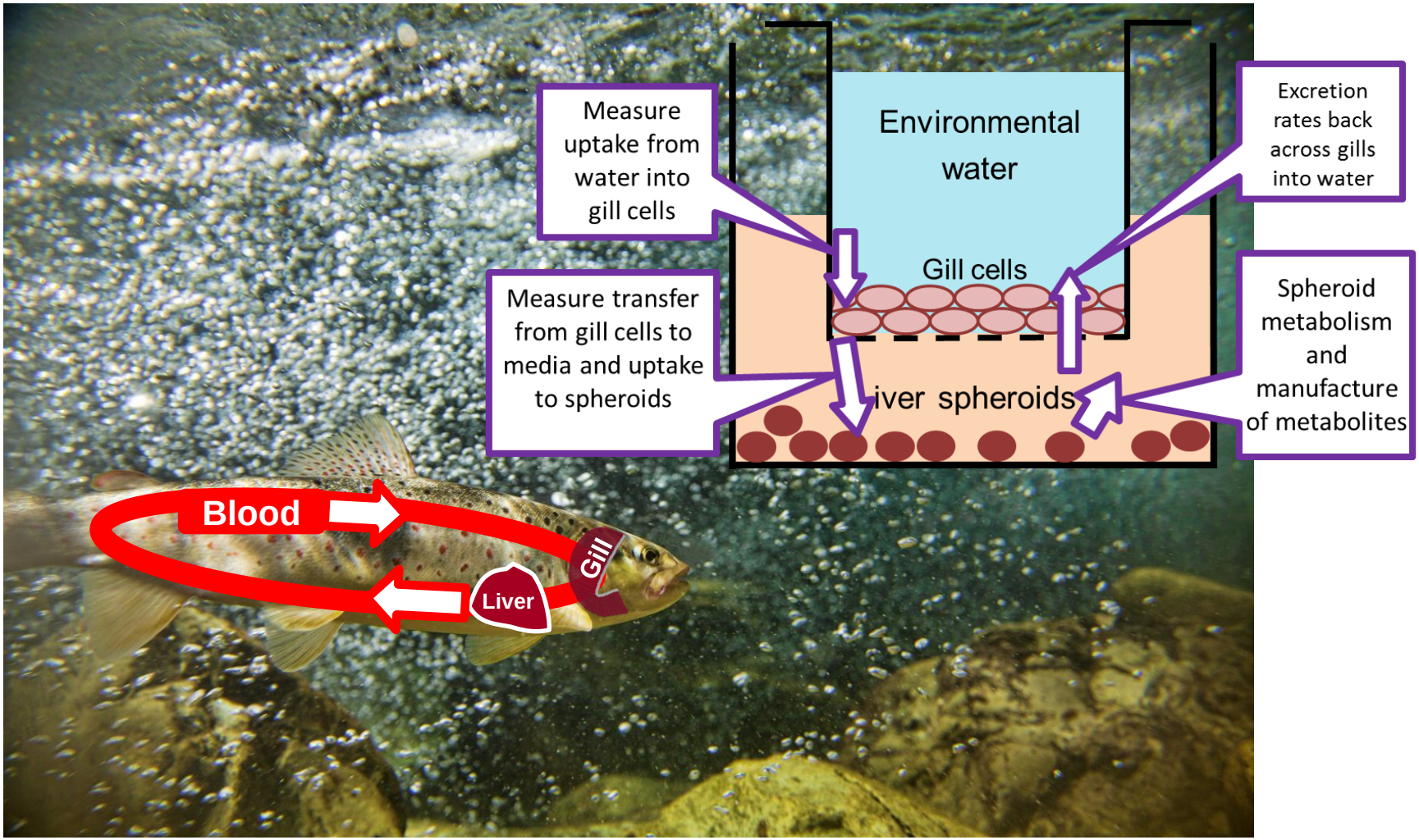
Gill layer production



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Gill layer production

Existing, well developed method exists;

See Schnell *et al.* (2016) Nature Protocols 11(3) 490-498
http://www.burylabs.co.uk/figcs/

PROTOCOL

Procedures for and experimen

Sabine Schnell[1,6], Lucy C Stott[1,6] Stewart F Owen[5] & Nic R Bury[1]

[1]Division of Diabetes and Nutritional Sciences, Zoology, University of British Columbia, Vancou Joint Research Centre, Institute of Environment Cheshire, UK. [6]These authors contributed equall ac.uk) or N.R.B. (nic.bury@kcl.ac.uk).

Published online 11 February 2016; doi:10.1038

This protocol describes how to recons supports within cell culture inserts. T monitoring and preparation for use in seeding of isolated gill cells twice ove technique. Approximately 5–12 d afte withstand freshwater on the apical ce alternative for toxicity testing, bioacc

ghts reserved.

INTRODUCTION

The freshwater fish gill is a multifun

Bury LABS

ABOUT | RESEARCH TOPICS | PUBLICATIONS | NEWS AND EVENTS | CONTACT

FiGCS – Fish Gill Cell Culture System

Home / FiGCS – Fish Gill Cell Culture System

The Fish Gill Cell Culture System (FiGCS) is a surrogate of the fish gill and been used as an in vitro tool to assess the uptake of chemicals from the water into the fish (Stott et al 2015). It may thus be of interest to industrial, pharmaceutical and governmental entities as a screen to assess the potential for chemicals to be taken up by fish as part of an environmental risk assessment.

The Benefits of the Fish Gill Cell Culture System include:

- Alternative, cheap and effective way of carrying out aquatic toxicology, physiology and endocrinology
- Fulfils obligations of the NC3r's – replace, reduce, refine the use of animals for scientific purposes
- Eliminates need to keep large stocks of live fish for testing
- Gill Cell Cultures from the gills of 2 fish effectively replaces 40-72 live fish using traditional testing methods
- Humane method – Using fish gill cell tissue means that no live fish are actually tested upon – lesser administrative burden on testing labs

This Fish Gill Cell Culture System cultures the primary gill cells of the freshwater rainbow trout on a flat permeable membrane. This procedure, known as the double seeded insert (DSI) technique, produces a heterogeneous gill epithelium

Browse all publications by topic

Aquatic Endocrinology | Aquatic Physiology | Aquatic Toxicology | Books | Fish Gill Cell Culture System | Government Report | News and Events

How the Fish Gill Cell Culture System Works

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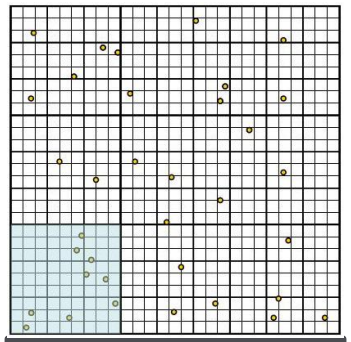
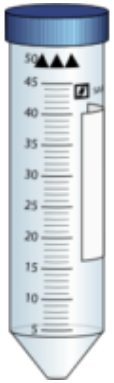
Gill layer production

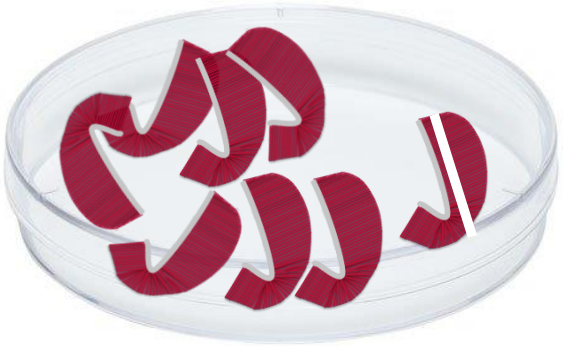


Haemocytometer count of cells

Wash & digest epithelial cells



Cut away filaments



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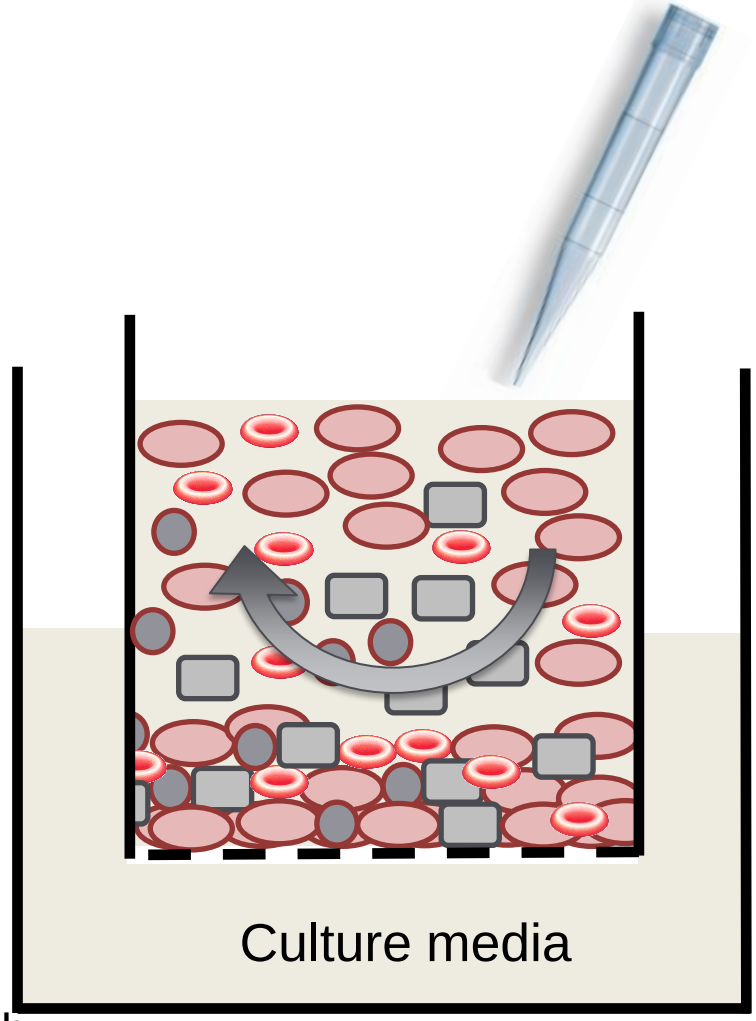
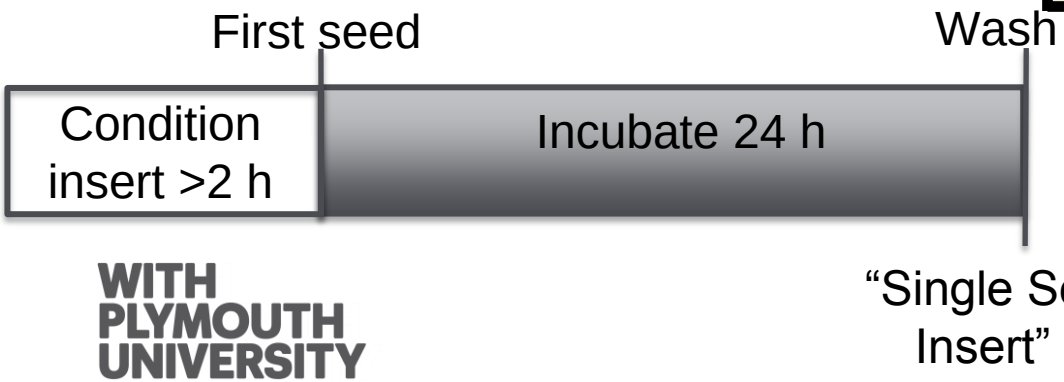
Gill layer production

1st seed of cells





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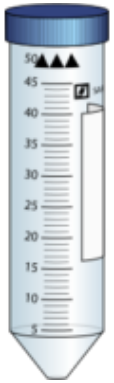
"Single Seeded Insert" SSI

Gill layer production

Prepare a 2nd set of epithelial cells

Cut away filaments

Wash & digest epithelial cells



Haemocytometer count of cells

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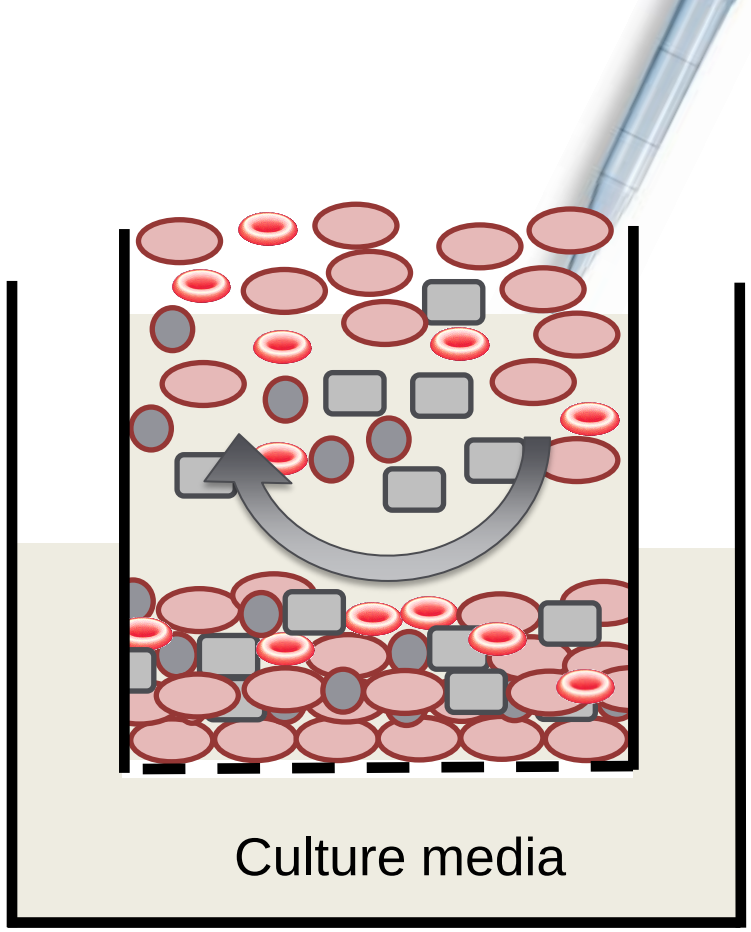
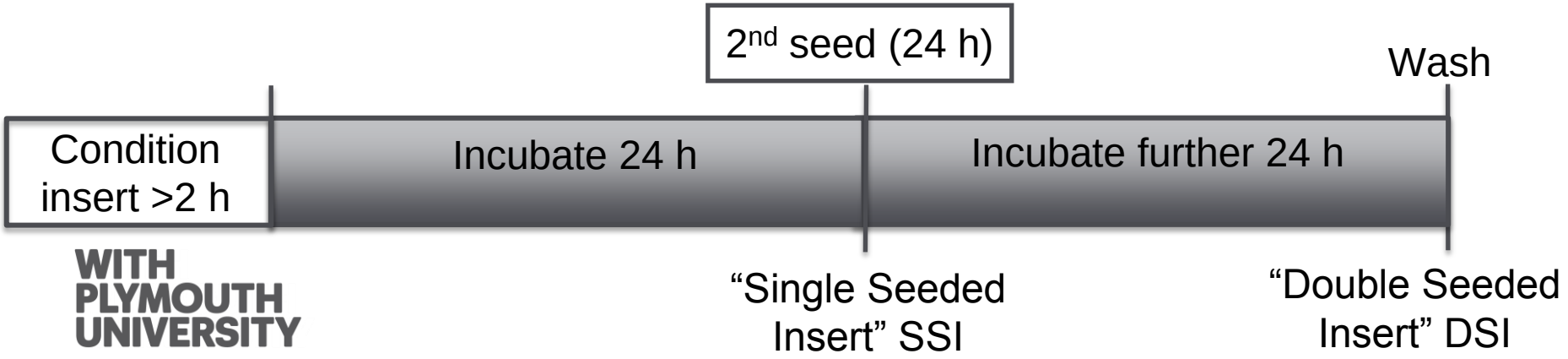
Gill layer production

2nd seed of cells

Higher diversity of cell types present in DSI

- Pavement cell
- Chloride cell
- Erythrocyte
- Goblet cell





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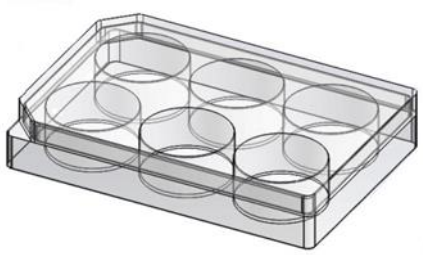
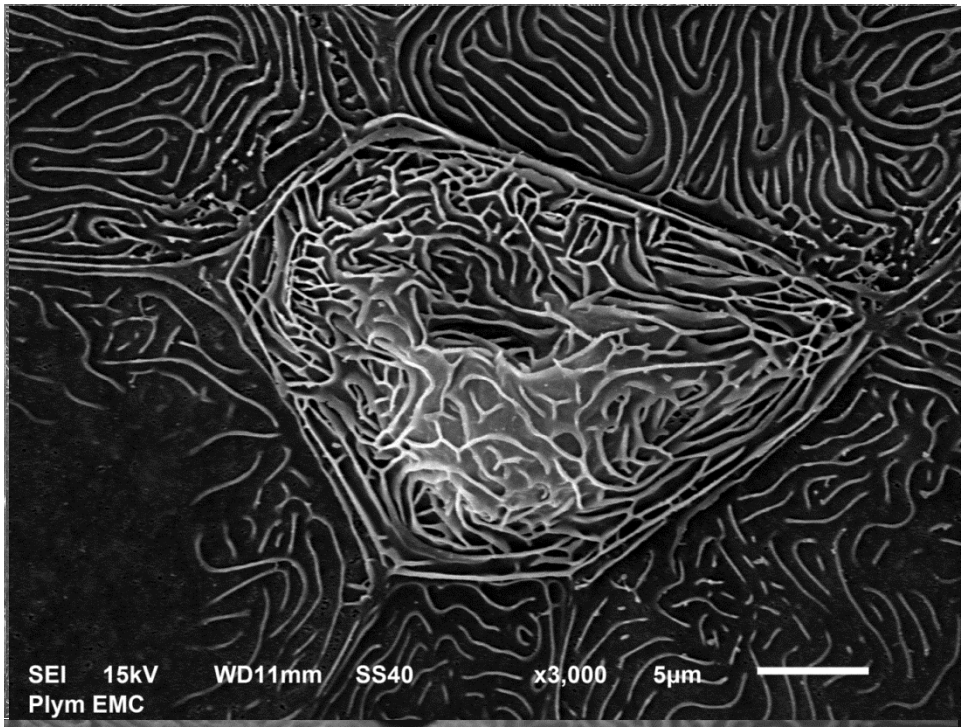
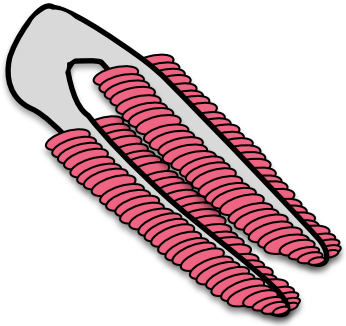
Gill layer production

SEM imaging:







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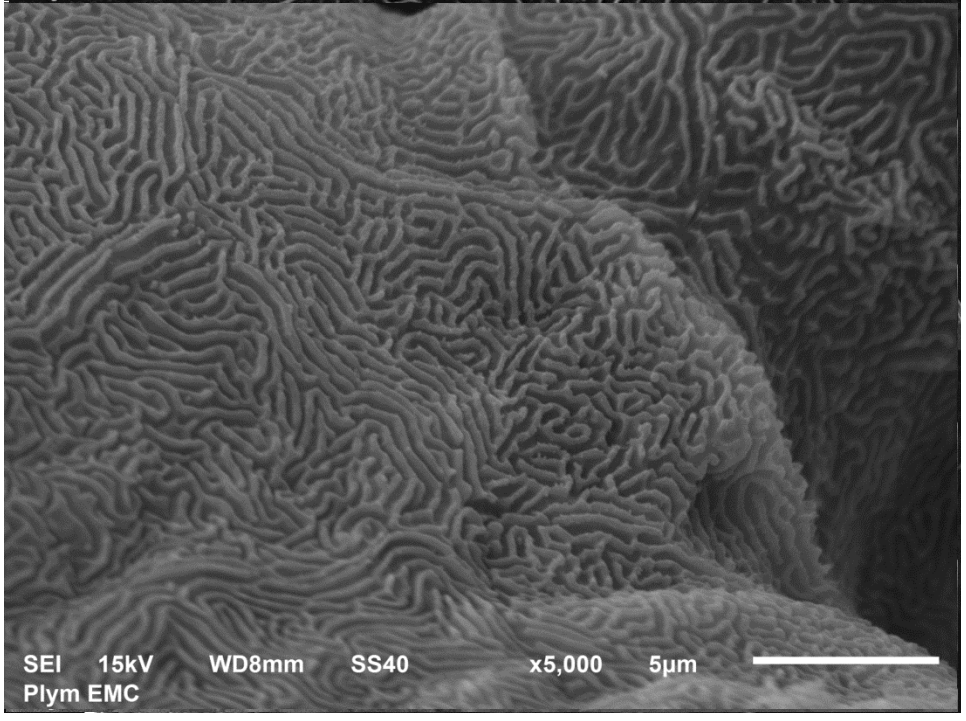
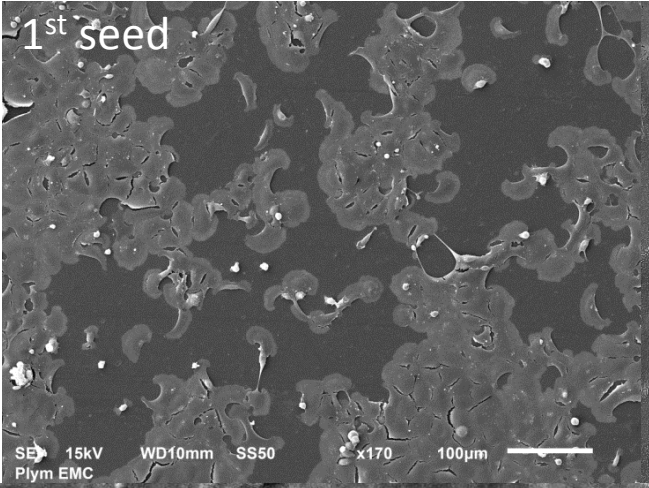
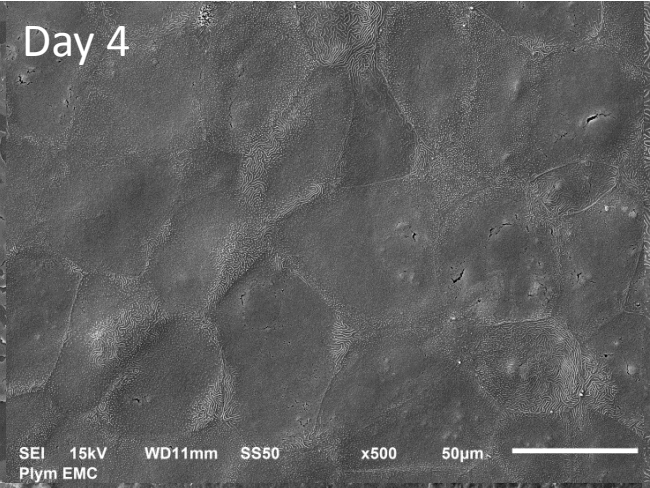
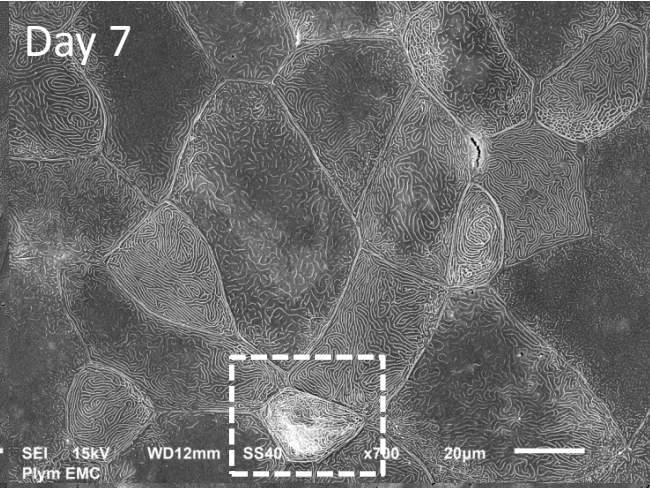
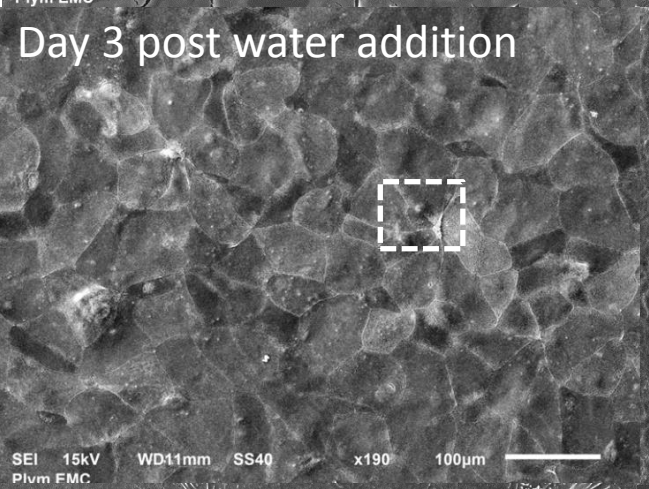
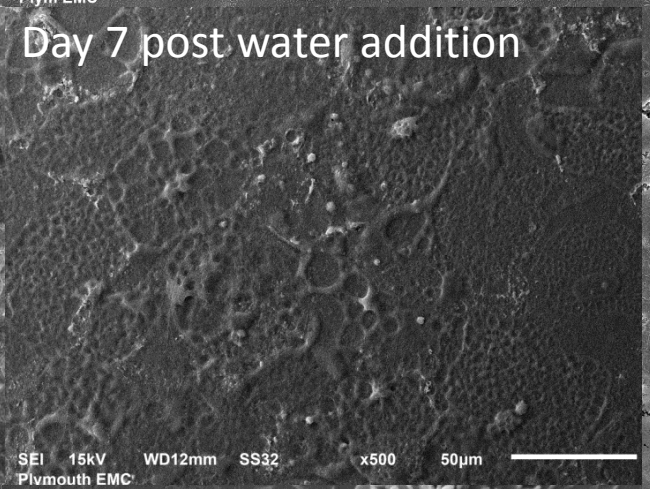


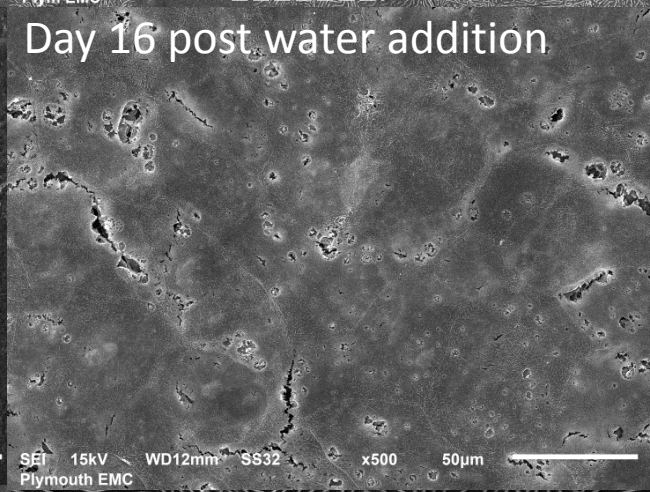

1st seed
SEI 15kV WD10mm SS50 x170 100µm
Plym EMC

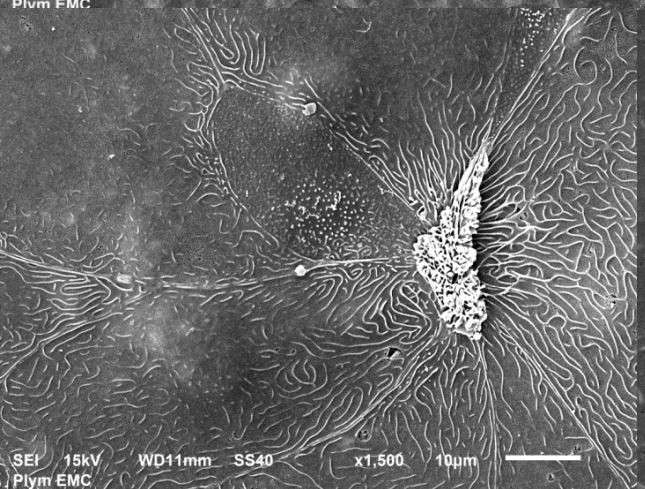

Day 4
SEI 15kV WD11mm SS50 x500 50µm
Plym EMC

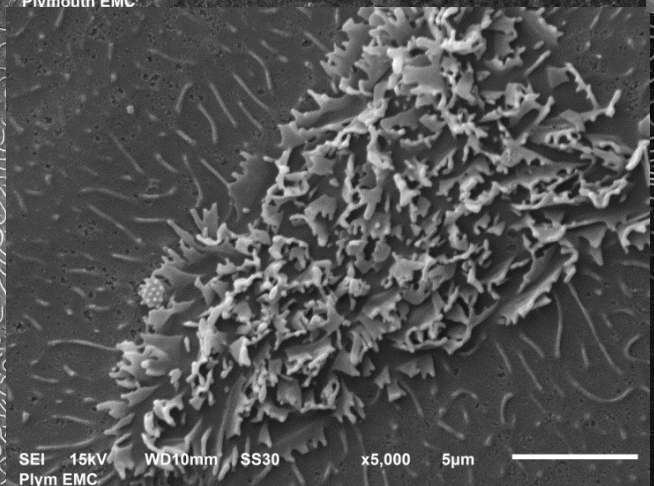

Day 7
SEI 15kV WD12mm SS40 x700 20µm
Plym EMC

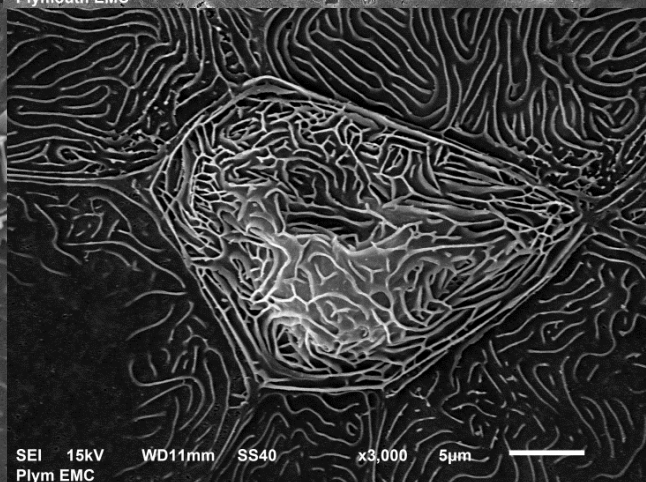

Day 3 post water addition
SEI 15kV WD11mm SS40 x190 100µm


Day 7 post water addition
SEI 15kV WD12mm SS32 x500 50µm
Plymouth EMC

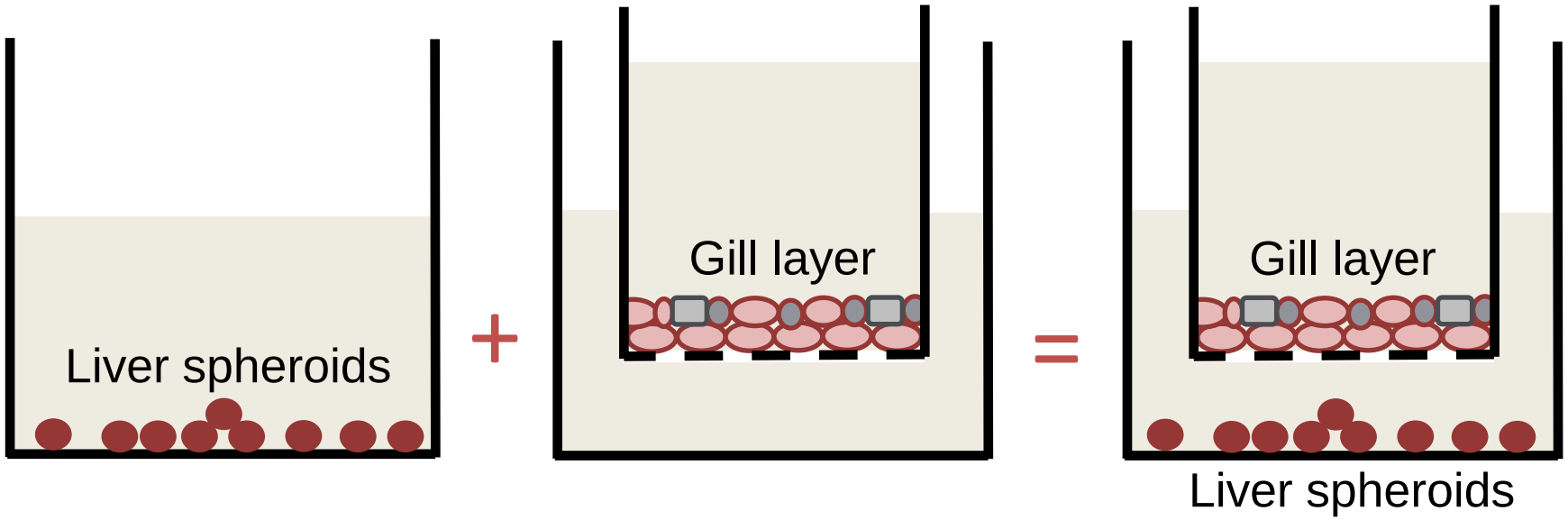

Day 16 post water addition
SEI 15kV WD12mm SS32 x500 50µm
Plymouth EMC


SEI 15kV WD11mm SS40 x1,500 10µm
Plym EMC


SEI 15kV WD10mm SS30 x5,000 5µm
Plym EMC


SEI 15kV WD11mm SS40 x3,000 5µm
Plym EMC

Co culture…



Easy..ish …for static / short term

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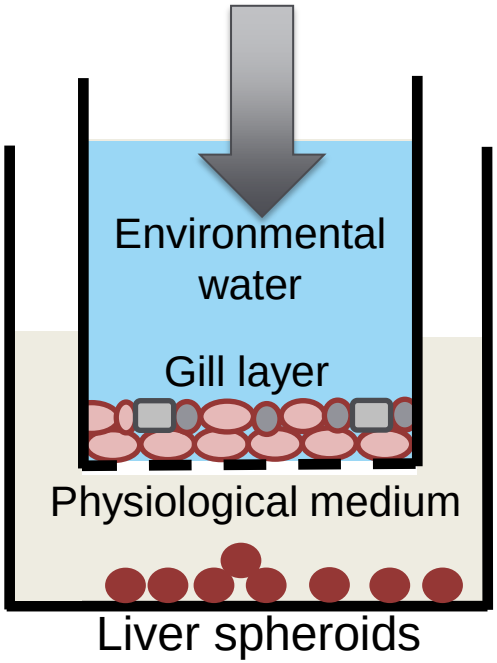
Co culture longer term

Challenges:

1. The swirling action is blocked by the insert.
2. Gill cultures only remain viable for ~2 days when exposed to water (to match *in vivo* situation)



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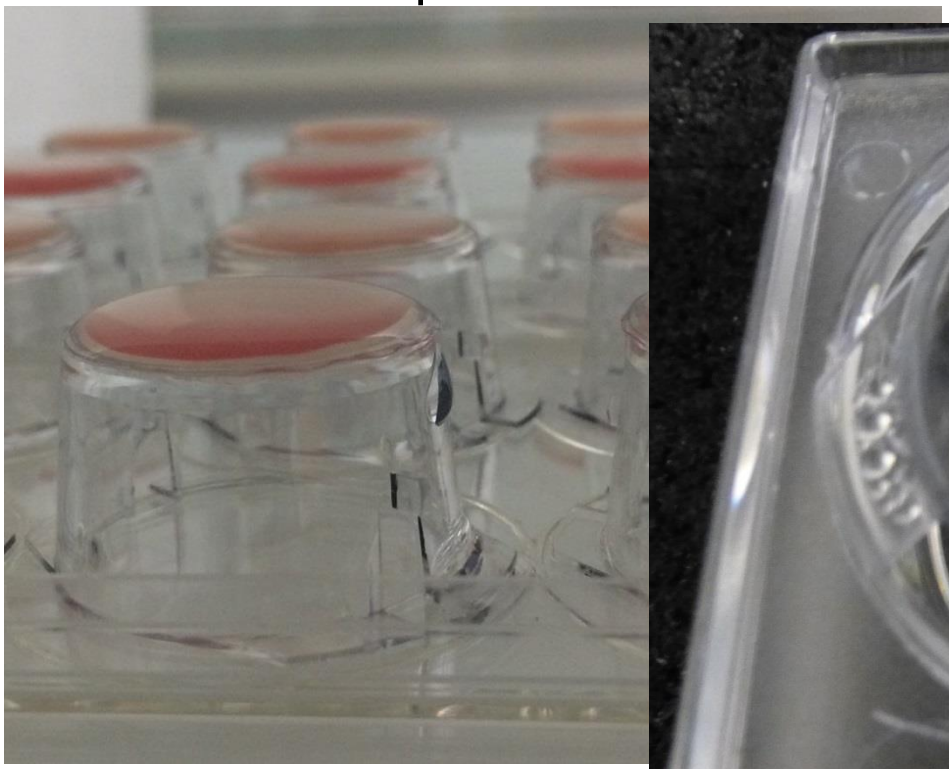
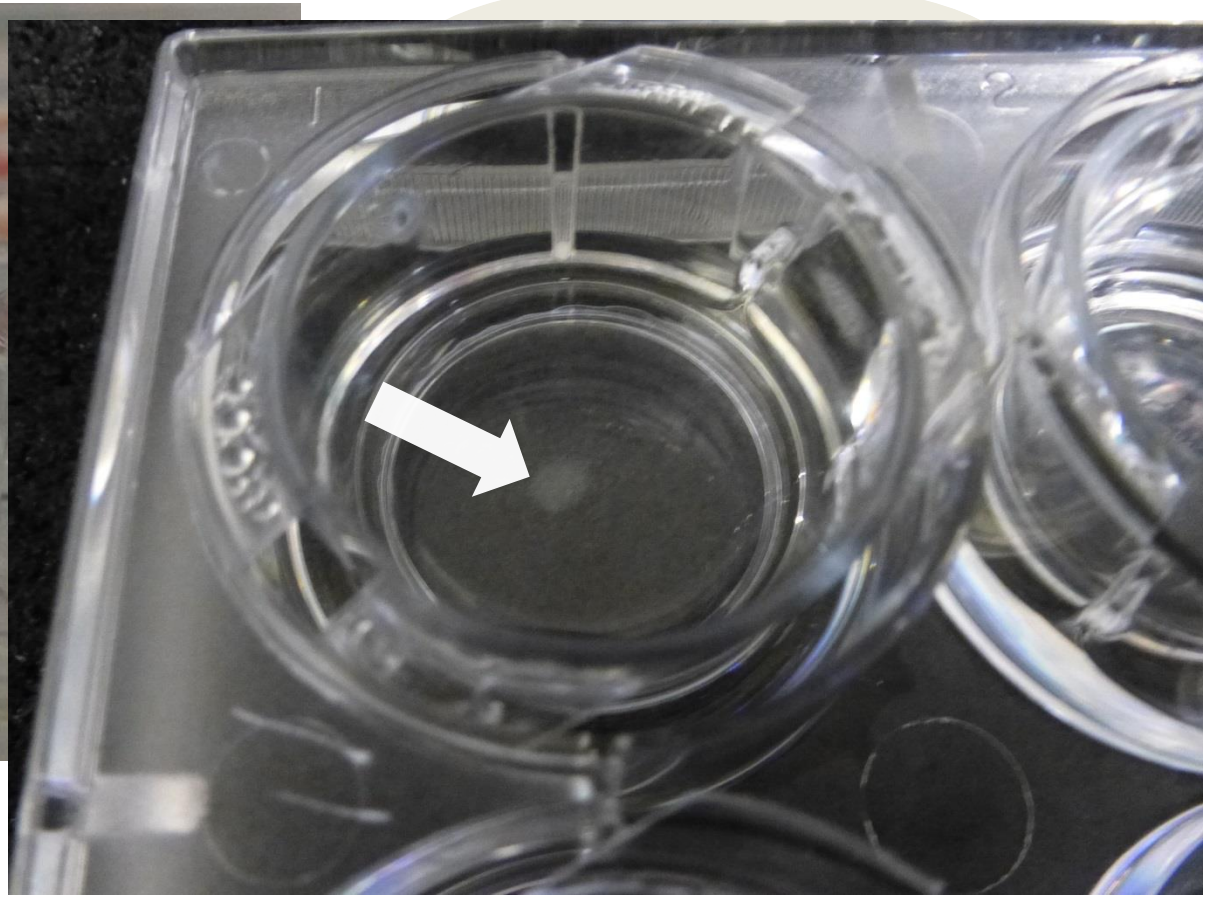
Co culture longer term

Issues:

1. The swirling action is blocked by the insert.

Solution: Upside-down culture?





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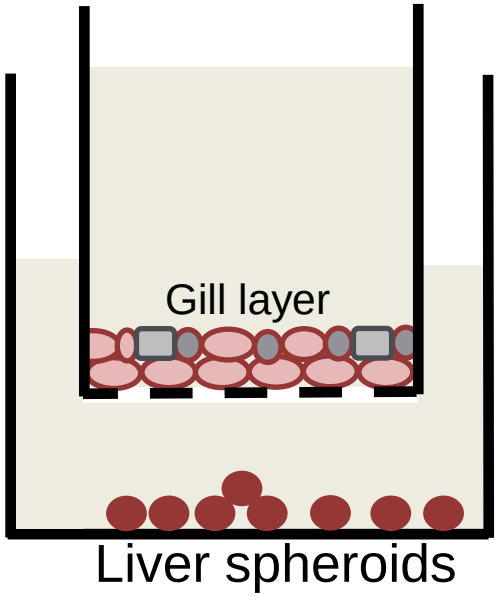
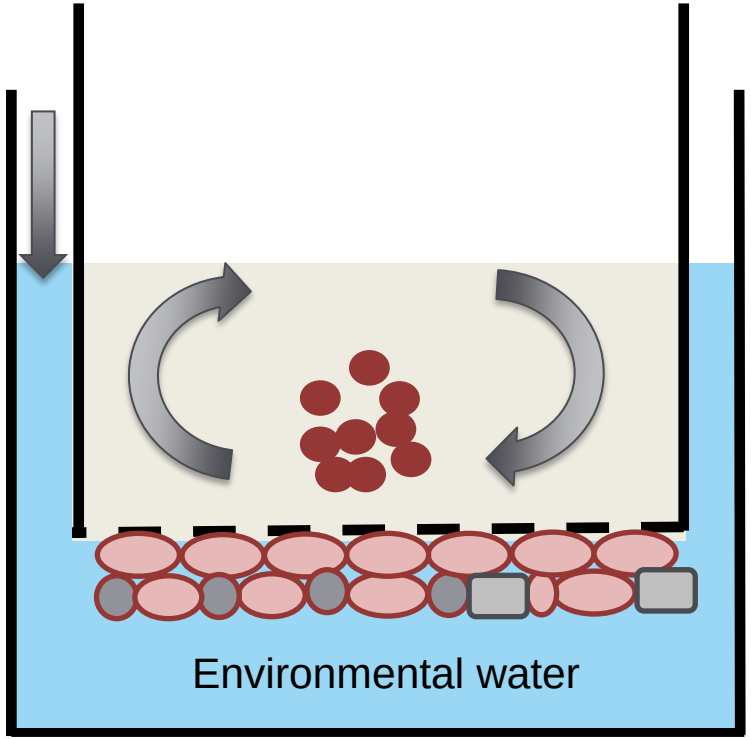
Co culture longer term

Issues:

1. The swirling action is blocked by the insert.

Solution: Upside-down culture?





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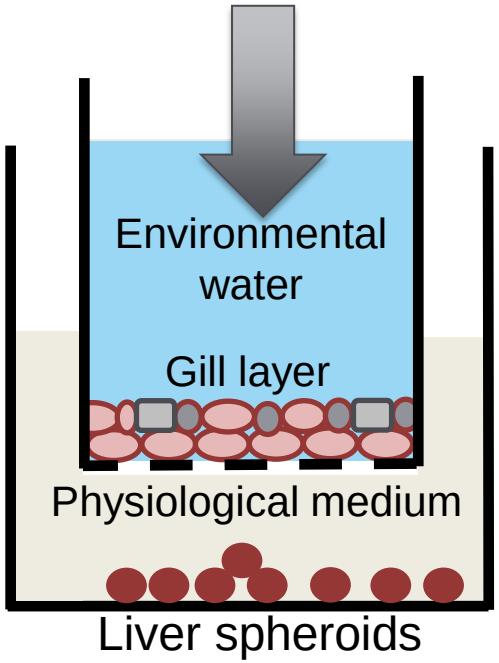
Co culture longer term

Issues:

1.
2. Gill cultures only remain viable for 2-3 days when exposed to water (to match *in vivo* situation)



See Poster tomorrow:

'Investigations to extend viability of a rainbow trout primary gill cell culture'

THPC09, Exhibition Hall, Thurs 0830

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Investigate 4 methodological alterations to existing protocol:

1. Membrane material
2. Gill pre-perfusion
3. Native serum supplementation
4. Larger inserts

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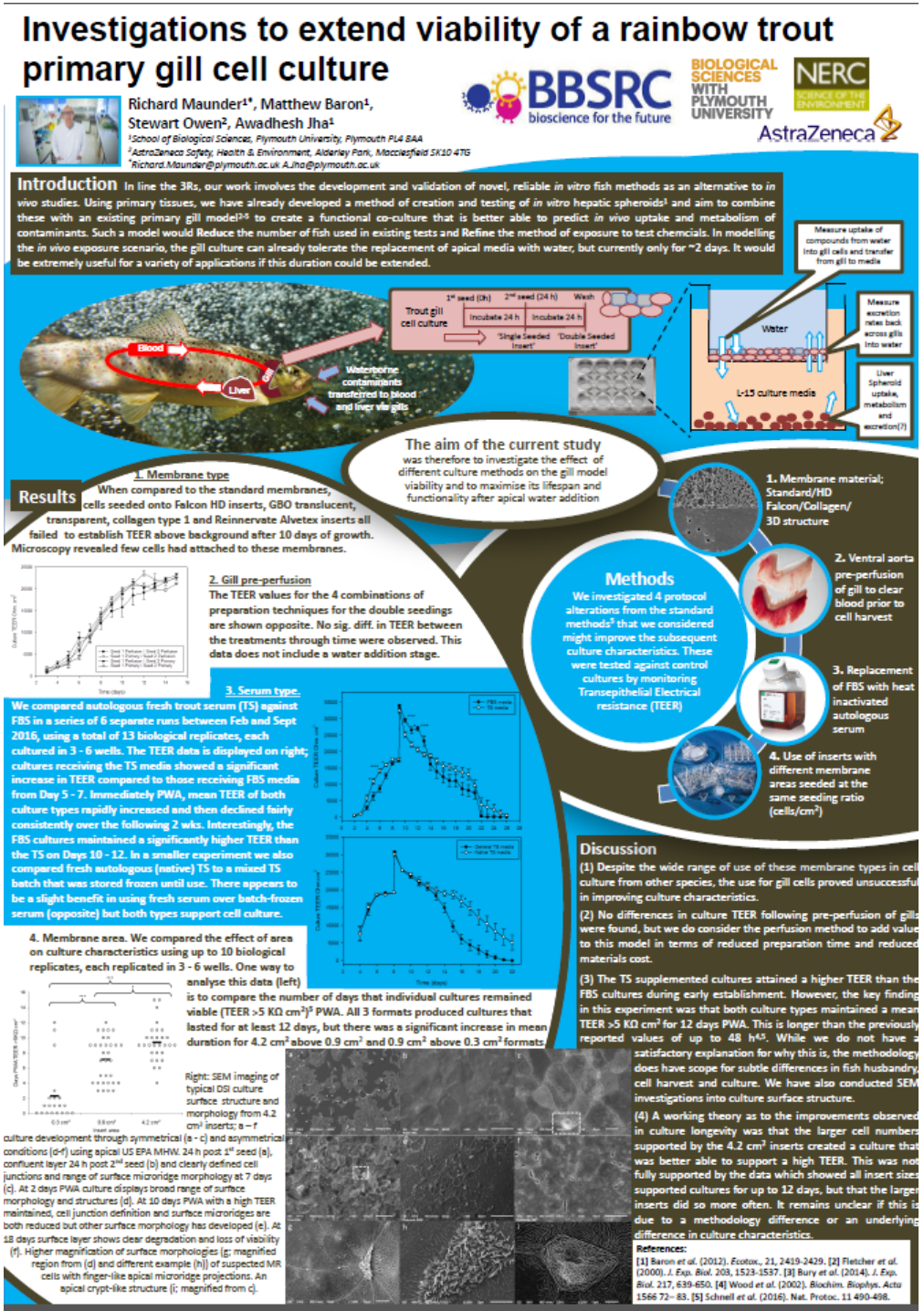

Ongoing / Future work

- Development and characterisation of single and co-culture models to suit specific exposure scenarios
  - Investigate communication between tissue types?
  - Can same fish supply multiple tissues?

- Single and co-culture exposures to various environmental contaminants (Pharmaceuticals / metals / PAHs)

- Analytical chemistry support to quantify concentrations in different compartments through time

- Development of Gut model for dietary uptake route…

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Thanks

Project team and staff at Plymouth University

Project funding:

RESEARCH WITH PLYMOUTH UNIVERSITY

NERC SCIENCE OF THE ENVIRONMENT

BBSRC bioscience for the future

AstraZeneca