Comparing the *in vitro* RTgutGC (Rainbow trout) intestinal cell line to native gastrointestinal tissue

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RESEARCH WITH PLYMOUTH UNIVERSITY

NERC SCIENCE OF THE ENVIRONMENT

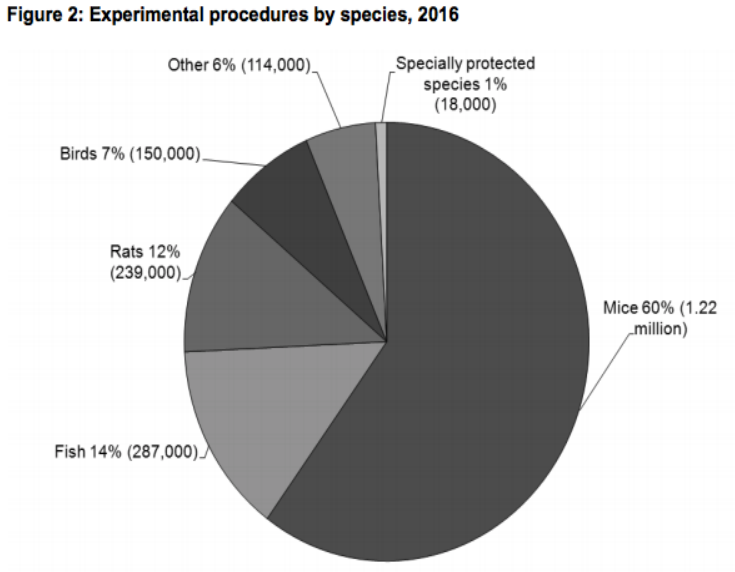
BBSRC bioscience for the future

Animals in the laboratory (UK)

- Animals (Scientific Procedures) Act 1986 (ASPA)
- Governed by the Animals in Science Regulation Unit

”..regulate the use of animals in scientific research for the benefit of people, animals and the environment through the provision of impartial licensing procedures and evidence based advice, and by encouraging the development and use of the 3Rs (replacement, reduction and refinement)”

- 5 % decrease in live animal usage (2015 to 2016) [1]
  - 206,000 procedural decrease since 2015



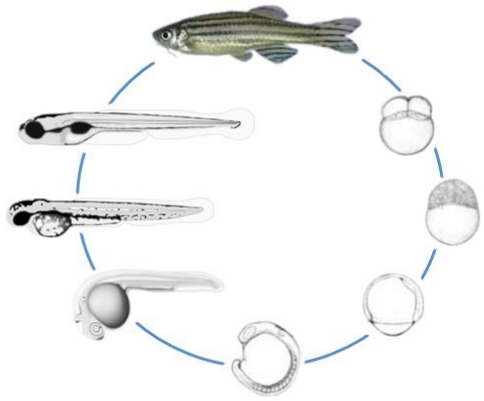
[1]Annual Statistics of Scientific Procedures on Living Animals Great Britain 2016

Importance of fish

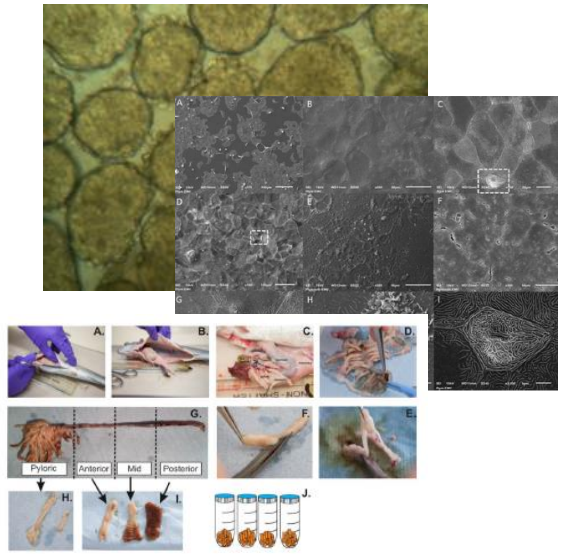
- As rodent research fell fish procedures increased
- Represent 14% of procedural animals
- Why used?
  - Chemicals produced by industry must be assessed under REACH legislation in Europe
    - UK (12,291 registrations of 5,674 substances) [1]
    - Germany (22,612 registrations of 10,313 substances) [1]
    - Austria (1169, registrations of 843 substance) [1]
  - Millions of fish could be used for bioaccumulation assessment along (pharmaceuticals)
  - Continued use of fish in research (physiology, disease, nutrition…)

[1]https://echa.europa.eu/registration-statistics-infograph#

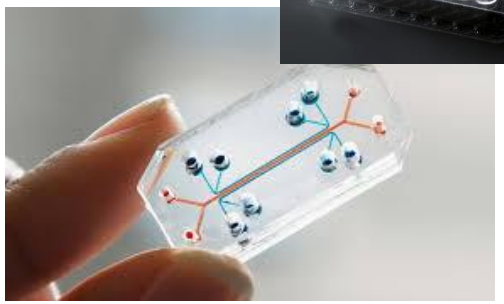
Alternative approaches to live fish



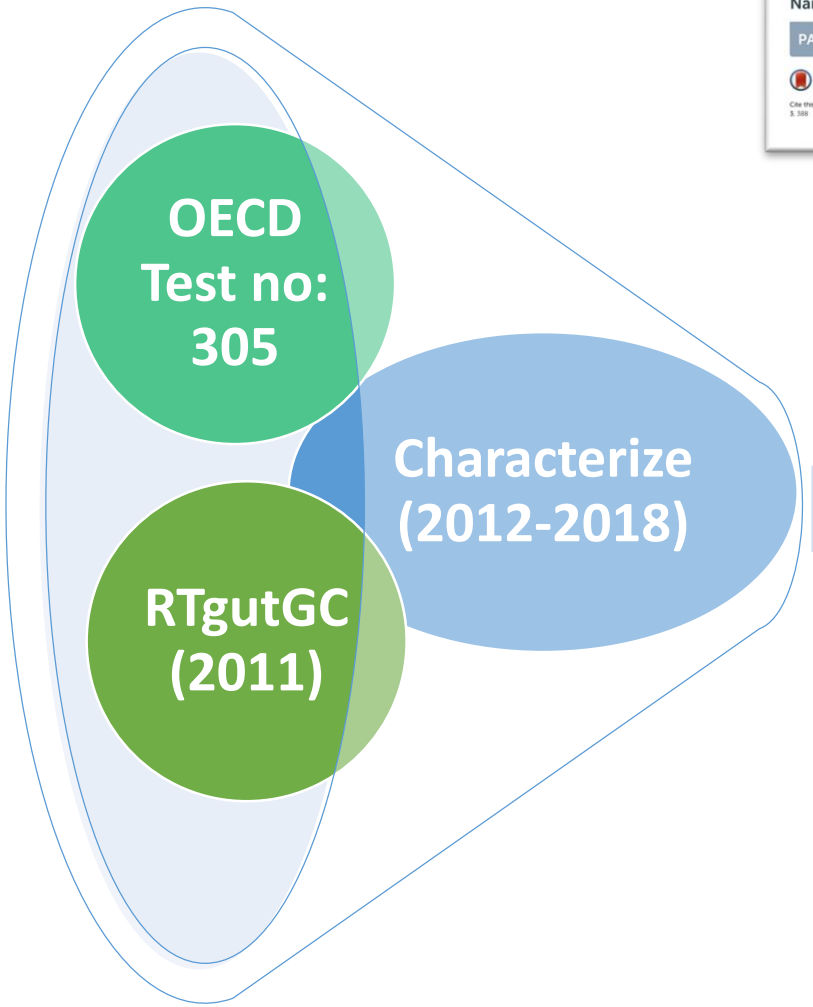
Early-life stage



Ex-vivo (primary cells)



In vitro



Environmental Science Nano

PAPER

A novel two-compartment barrier model for investigating nanoparticle transport in fish intestinal epithelial cells†

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Effect of acid blue 80, an anthracenedione dye, on rainbow trout liver, gill and gut cells *in vitro*

P.N. Catherine Tee[a], Y.T. Janice Wong[a], James P. Sherry[b], Niels C. Bols[a,*]

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

A comparison of rainbow trout cell lines for their expression of the major histocompatibility complex genes and the induction of beta-2-microglobulin by dsRNA

Atsushi Kawano[a], Stephen C. Kales[a], Kazuhiro Fujiki[a], Stephanie J. DeWitte-Orr[a], Brian Dixon[a], Lucy F.L. Lee[b], Niels C. Bols[a,*]

ACS APPLIED MATERIALS & INTERFACES

Research Article

www.acsami.org

Ultrathin Alumina Membranes as Scaffold for Epithelial Cell Culture from the Intestine of Rainbow Trout

Carolin Drieschner,[†,‡] Matteo Minghetti,[†,§] Songmei Wu,[‡,∥] Philippe Renaud,[‡] and Kristin Schirmer[*,†,⊥,▽]

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Constitutive mRNA expression and protein activity levels of nine ABC efflux transporters in seven permanent cell lines derived from different tissues of rainbow trout (*Oncorhynchus mykiss*)

Stephan Fischer[a,b,c], Jovica Loncar[d], Roko Zaja[d], Sabine Schnell[e], Kristin Schirmer[b,c,f], Tvrtko Smital[d], Till Luckenbach[a,*]

Cell Biol Toxicol
DOI 10.1007/s10565-017-9385-x

A fish intestinal epithelial barrier model established from the rainbow trout (*Oncorhynchus mykiss*) cell line, RTgutGC

Matteo Minghetti · Carolin Drieschner · Nadine Bramaz · Hannah Schug · Kristin Schirmer

Mutat Res Gen Tox En

journal homepage: www.elsevier.com/locate/gentox

Assessing the impact of benzo[*a*]pyrene with the *in vitro* fish gut model: An integrated approach for eco-genotoxicological studies

Laura M. Langan[a], Silvia Arossa[a], Stewart F. Owen[b], Awadhesh N. Jha[a,*]

Ecotoxicology
DOI 10.1007/s10646-017-1838-8

Application of the rainbow trout derived intestinal cell line (RTgutGC) for ecotoxicological studies: molecular and cellular responses following exposure to copper

Laura M. Langan[1] · Glenn M. Harper[2] · Stewart F. Owen[3] · Wendy M. Purcell[4] · Simon K. Jackson[4] · Awadhesh N. Jha[1]

Fish & Shellfish Immunology 44 (2015) 138–146

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

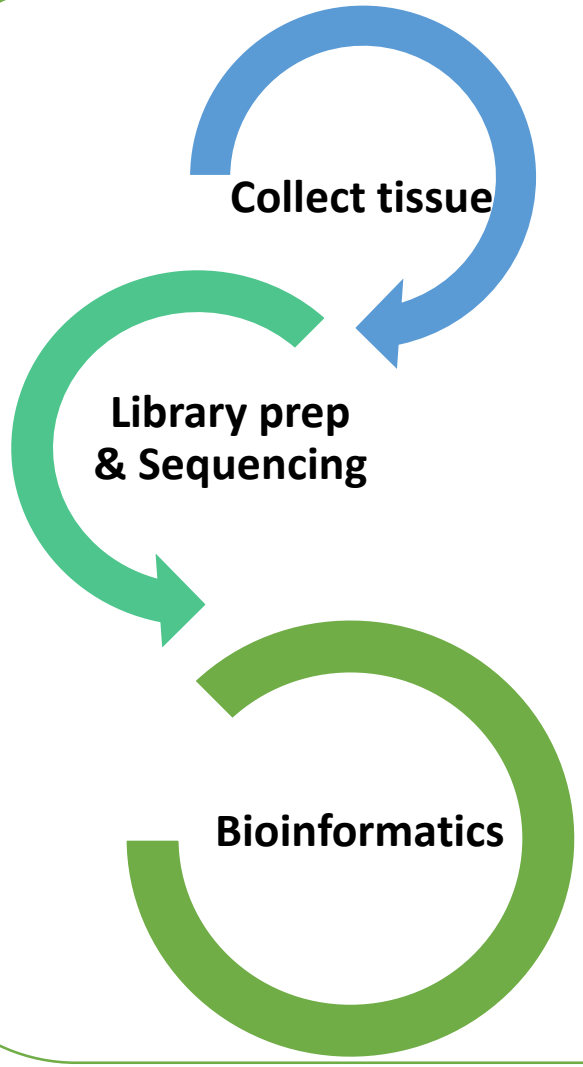
Class-A scavenger receptor function and expression in the rainbow trout (*Oncorhynchus mykiss*) epithelial cell lines RTgutGC and RTgill-W1

Sarah J. Poynter, Jeremy Weleff, Adam B. Soares, Stephanie J. DeWitte-Orr*

- **Numerous studies use the RTgutGC as a toxicology model**
  - However, minimum knowledge of how much model deviates from origin tissue

- **Aim is to characterize the transcriptome of the RTgutGC cell line**
  - Identify large number of genes/targets
  - Mechanism of action
  - Eventually identify potential mode of action of compound based on physiological and transcriptome similarity to origin tissue using replacement mode

Experimental design and analysis



- Isolate RNA from epithelial cells grown to 70% confluence (non-parallel passages)
- Extract RNA (RNAeasy mini kit, Qiagen)
- Assess quality & Quantity (Nano drop & RIN)
- Pool samples (n= 5)

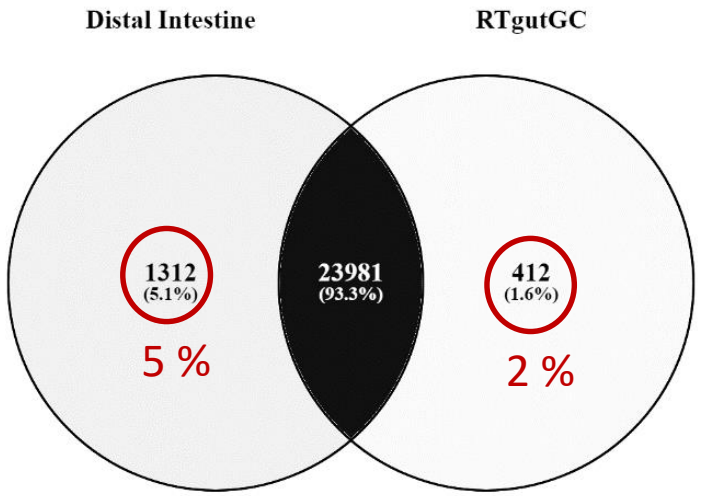
- Carried out by Earlham Institute (UK)
- TruSeq RNA protocol using Illumina

- Quality control (FastQC)
- Alignment to rainbow trout genome (RSEM & Bowtie)
- Filtering based on CPM (Counts Per Million) and sensitivity plots
- Filter results blasted against NR database (Dec 2017)
- Further summary analysis (BLAST2GO)
- Differential expression and graphing R packages (NOISeq)
- GSEA (ClusterProfiler)

Results:

Alignment

- 79,245 transcripts following alignment (REFSEQ)
- Filter based on CPM > 5
- RPKM normalisation
- 25,705 transcripts



Differentially expressed genes (DEG)

DEG in the cell line compared to the tissue

- **Unmodified data**
  - **17855 DEG**
  - **8454 (up) + 9401 (down)**
- **Removal of duplicated Gene ID's**
  - **12,014 DEG**
  - **5709 (up) + 6305 (down)**

Top 10 DEG

Up-regulated

1	Ferritin, middle subunit-like
2	Heat shock cognate protein 70a
3	Ferritin H-2
4	Ferritin H-1
5	Elongation factor EF1-alpha
6	Ferritin H-3
7	Simple type II keratin K8b (S2)
8	Glutathione S-transferase P-like
9	Lipoprotein lipase-like
10	Neuroblast differentiation-associated protein (AHNAK-like)

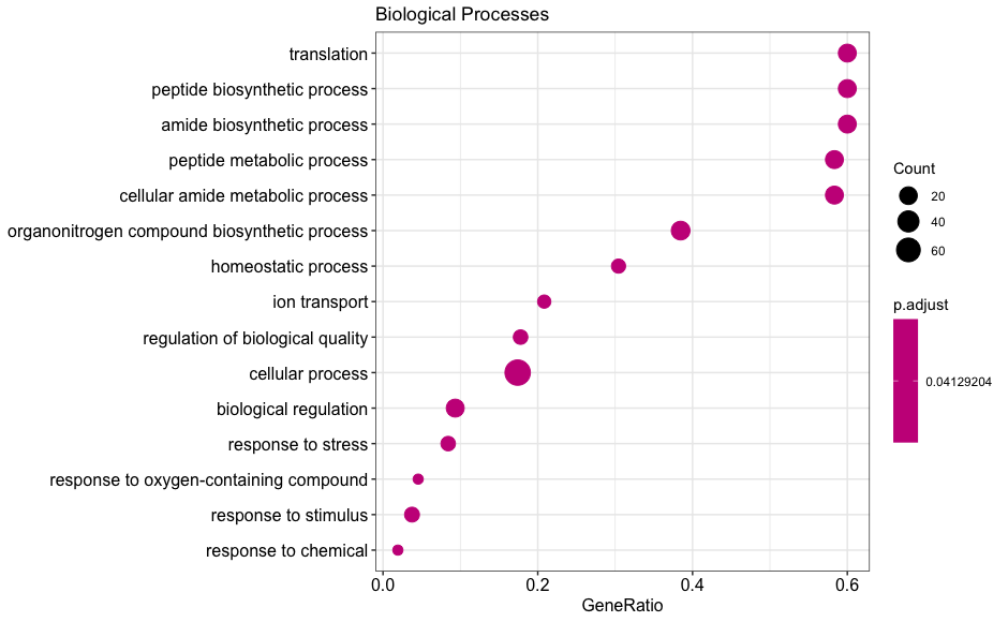
Down-regulated

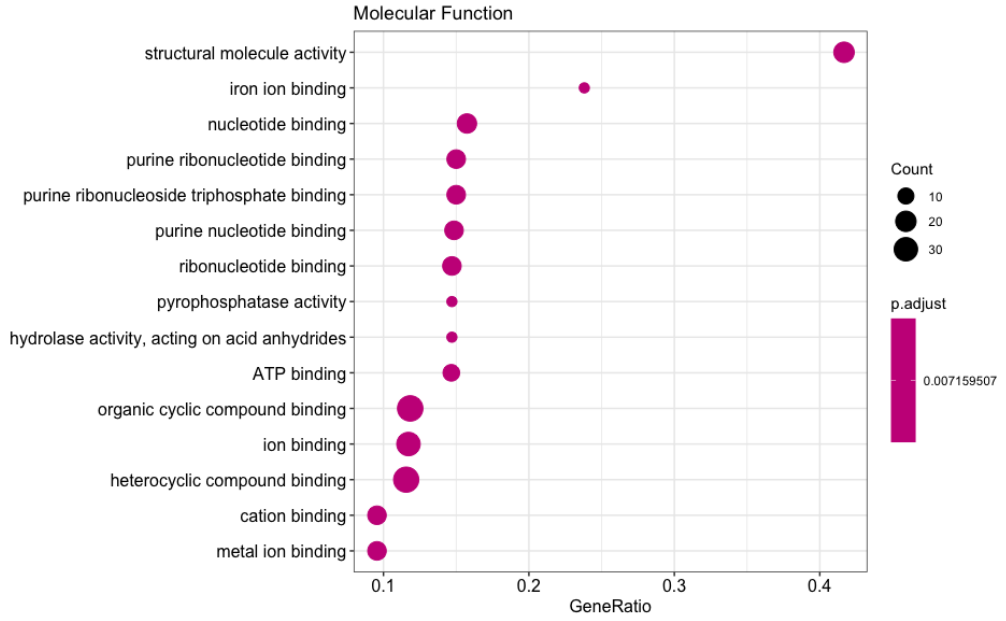
1	Cathepsin L1-like
2	Uncharacterized LOC110528711
3	Fatty acid binding protein 2
4	Apolipoprotein B-100-like
5	Actin, cytoplasmic 1
6	Uncharacterized LOC110494266
7	UPF0183 protein C16orf70 homolog
8	Cadherin related family member 2
9	Galectin-2-like
10	Mannan-binding lectin H2

Gene set enrichment analysis (GSEA)

"Method to identify classes of genes or proteins that are over-represented in a large set of genes or proteins"

- GSEA performed on full list of transcripts of the RTgutGC (Following removal of 0 count sequences)
  - 46,023 transcripts
  - Limited to top 15 biological processes and molecular function





GO-Annotations

Molecular function

1. Organic cyclic compound binding
2. Heterocyclic compound binding
3. Ion binding
4. Protein binding
5. Hydrolase activity
6. Small molecular binding
7. Carbohydrate derivative binding
8. Transferase activity
9. Catalytic activity (acting on protein)
10. DNA binding transcription factor activity

Biological Processes

1. Organic substance metabolic process
2. Primary metabolic process
3. Cellular metabolic process
4. Nitrogen compound metabolic process
5. Biosynthesis process
6. Regulation of biological process
7. Regulation of cellular process
8. Response to chemical
9. Cellular response to stimulus
10. Response to stress

Conclusions

- By describing and mining the gene profile of the only fish intestinal cell line, this study has:
  - For the first time, established comparability to native tissue
    - Facilitated informed experimental design in toxicology using this cell line
  - Aided in identification of functional and pathway annotation (*Ongoing*)
  - Aided in genomic studies in aquatic cell lines
  - Provided code for analysis so can be replicated in the future (*Ongoing*)
  - Developed a genetic toolkit which others can help shape 3Rs research using aquatic cell lines

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