Institution: University of Portsmouth (UoP)

Unit of Assessment: UoA3 - Allied Health Professions, Dentistry, Nursing and Pharmacy

Title of case study: The European *Xenopus* Resource Centre: supporting the 3Rs, enhancing the efficiency of biomedical research and improving the diagnosis of rare genetic diseases

Period when the underpinning research was undertaken: 2010 - 31 December 2020

Details of staff conducting the underpinning research from the submitting unit:

Name(s):	Role(s) (e.g. job title):	Period(s) employed by submitting HEI:
Prof Matt Guille	Professor of Developmental Genetics	01/12/1995 - date
Dr Colin Sharpe	Senior Lecturer	01/01/2000 - date

Period when the claimed impact occurred: 01/08/2013 - 31/12/2020

Is this case study continued from a case study submitted in 2014? N

1. Summary of the impact

The EXRC at the UoP provides quality-assured frog lines, reagents and services to over 180 laboratories worldwide and is a lead for the *Xenopus* community. Since 2013, it has developed methods and approaches that have reduced and refined the use of frogs in experimentation and driven the incorporation of '3Rs' in animal research into policy and practice. EXRC research and activities have improved the efficiency and effectiveness of the global *Xenopus* research base and enhanced the capability and competitiveness of international companies supporting European chemical regulation. Research to clarify variants of unknown significance associated with rare genetic disorders has transformed the speed of diagnosis and provided clinicians with the knowledge to develop targeted interventions for treatment or management.

2. Underpinning research

The clawed frog (*Xenopus laevis* and *X. tropicalis*) is an essential vertebrate model for biomedical research. Long established in developmental and cell biology, more recent mapping of *Xenopus* genome sequences (Hellsten et al, 2010; Session et al, 2016), and their remarkable structural similarity to the human genome, means that findings from *Xenopus* can be used to model many human conditions and diseases. As a result, the international *Xenopus* community has expanded to include clinical scientists and human geneticists. *Xenopus*-based research has been awarded the Nobel Prize in Physiology or Medicine twice in the last 15 years. The increased use of the *Xenopus* model and the creation of large numbers of inbred, mutant and transgenic *Xenopus* lines necessitated the creation of globally-centralised *Xenopus* stock centres.

In 2006, and supported by funding from the Wellcome Trust (**G1**, **G3**, **G5**) and the BBSRC (**G2**, **G4**), the European *Xenopus* Resource Centre (EXRC) was established at the UoP. Led by **Professor Matt Guille** and **Dr Colin Sharpe**, researchers at the EXRC have developed a suite of approaches that have **reduced the numbers of adult *Xenopus* required to support research programmes, improved *Xenopus* care and more efficiently used those that are kept.** These achievements are in line with the principles of the '3Rs' in animal research: reduction, refinement, and replacement, and include:

- housing and supplying mutant and transgenic frog lines, thus enabling laboratories to purchase animals when needed, rather than housing a critical mass of each line they use;
- developing refined husbandry and breeding protocols to support the establishment of colonies of *X. tropicalis*, whose sequenced, diploid genome and rapid growth make genetic approaches to biomedical research increasingly practical (**R1**);
- confirming there was no significant potential for the spread of transmittable amphibian disease through the co-occurrence of laboratory *Xenopus* with native species (**R2**);
- developing protocols for the supply of oocytes, egg extracts and embryos directly to users, reducing both the movement of live animals and the need to maintain local colonies;
- developing robust methods for improving *Xenopus* sperm recovery and cryopreservation (**G6**, **G7**). This research demonstrated that 32 fertilisations could be performed with frozen sperm from one male (c.f. 2-8 when fresh sperm are used) (**R3**) and the methods offer the potential to eliminate the need to transport male frogs, significantly reduce the number of male frogs required for research and refine their husbandry;
- developing novel, tissue-specific transgenic lines of *Xenopus* (**R4**);
- developing protocols for raising and purifying frog-specific antibodies (**R5**).

The EXRC continues to develop and apply *Xenopus* technologies in response to new techniques and to address novel questions. Since 2018, the EXRC research team have worked with clinical geneticists and computational genome scientists at the University of Southampton and the Wessex NHS Genomic Medicine Centre, as part of the Genomics England 100,000 Genomes Project. Proof of principle work has demonstrated the efficiency of CRISPR/Cas gene edited *Xenopus* models for screening variants of unknown significance found in clinical genomic testing and associated with rare genetic disorders (RGDs). This work has already identified new disease-causing genes: e.g. COPB1 (brain disorders and cataracts), GRIA1 (seizures); and a new disease-causing change in the TRIO gene (intellectual disability and autism spectrum disorders) (**R6**). Significantly, results from diagnostic tests using *Xenopus* are available within a month, compared with up to a year using mouse models. The Portsmouth and Southampton teams have recently been awarded GBP992,000 from the Medical Research Council to scale up the pipeline of gene-function analysis in *Xenopus*.

3. References to the research

3.1. Research outputs

R1. Jafkins, A., Abu-Daya, A., Noble, A., Zimmerman, L. B., & **Guille, M.** (2012). Husbandry of *Xenopus tropicalis*. In S. Hoppler, & P. D. Vize (Eds.), *Xenopus protocols: post-genomic approaches* (2nd ed. ed., pp. 17-31). (Methods in molecular biology; No. 917). Humana Press. https://doi.org/10.1007/978-1-61779-992-1_2

R2. Tinsley, R. C., Coxhead, P. G., Stott, L. C., Tinsley, M. C., Piccinni, M. Z., & **Guille, M. J.** (2015). Chytrid fungus infections in laboratory and introduced *Xenopus laevis* populations: assessing the risks for U.K. native amphibians. *Biological Conservation*, *184*, 380-388. https://doi.org/10.1016/j.biocon.2015.01.034

R3. Pearl, E., Morrow, S., Noble, A., Lerebours, A., Horb, M., & **Guille, M.** (2017). An optimized method for cryogenic storage of *Xenopus* sperm to maximise the effectiveness of research using genetically altered frogs. *Theriogenology*, *92*, 149-155. https://doi.org/10.1016/j.theriogenology.2017.01.007

R4. Love, N. R., Thuret, R., Chen, Y., Ishibashi, S., Sabherwal, N., Paredes, R., Alves-Silva, J., Dorey, K., Noble, A. M., **Guille, M. J.**, Sasai, Y., Papalopulu, N., & Amaya, E. (2011). pTransgenesis: a cross-species, modular transgenesis resource. *Development, 138*(24), 5451-5458. https://doi.org/10.1242/dev.066498

R5. Piccinni, M. Z., & **Guille, M. J.** (2020, Sep 1). Raising antibodies for use in *Xenopus*. (9 ed.) Cold Spring Harbor Laboratory Press. https://doi.org/10.1101/pdb.prot105585

R6. Barbosa, S., Greville-Heygate, S., Bonnet, M., Godwin, A. L., Fagotto-Kaufmann, C., Kajava, A. V., Laouteouet, D., Mawby, R., Wai, H. A., Dingemans, A., De Vries, B., Willems, M., Capri, Y., Mehta, S. G., Cox, H., Goudie, D., Vansenne, F., Turnpenny, P., Vincent, M., ... Baralle, D. (2020). Opposite modulation of RAC1 by mutations in *TRIO* is associated with distinct, domain specific neurodevelopmental disorders. *American Journal of Human Genetics*, *106*(3), 338-355. https://doi.org/10.1016/j.ajhg.2020.01.018

3.2. Evidence for the quality of the research

The research outlined above has been published in high quality, peer-reviewed international journals and supported by competitive peer-reviewed awards from the Wellcome Trust, UK Research Councils (BBSRC, NERC) and NC3Rs (with **Guille** and/or **Sharpe** as Principal Investigators). R6 is submitted in REF2 with Output ID 24932843.

3.3. Related grants

G1. **Guille, M.J.** *A European Stock Centre for Xenopus*. Funded by Wellcome Trust, 01/09/2006 - 31/08/2011 (GBP1,472,290)

G2. Jones, E.A. & **Guille, M.J.** *The European Xenopus Stock Centre: a bioinformatically integrated molecular and animal resource*. Funded by BBSRC, 10/2008 - 10/2011 (GBP404,628)

G3. **Guille, M.J.** & **Sharpe, C.** *The European Xenopus Resource Centre*. Funded by Wellcome Trust, 01/09/2011 - 31/08/2013 (GBP454,472)

G4. **Guille, M.J.**, Allan, V. & **Sharpe, C.** *Molecular and Bioinformatic support for the European Xenopus Resource Centre*. Funded by BBSRC, 01/09/2013 - 31/08/2018 (GBP576,840) and 01/09/2018 - 31/08/2023 (GBP694,313)

G5. **Guille, M.J.** & **Sharpe, C.** *The European Xenopus Resource Centre (EXRC)*. Funded by Wellcome Trust, 01/09/2013 - 31/08/2018 (GBP1,374,932) and 01/09/2018 - 31/08/2023 (GBP1,551,649)
G6. **Guille, M.J.** *Genomic aspects of DNA damage induced by germplasm cryopreservation*. Funded by NERC, 10/2011 - 10/2015 (GBP67,307)
G7. **Guille, M.J.** & **Sharpe, C.** *Reducing the use and refining the distribution of male Xenopus.* Funded by NC3Rs, 09/2016 - 08/2018 (GBP106,760).

4. Details of the impact

The EXRC is the largest of three centres worldwide that provide biological and bioinformatic resources for studying *Xenopus*. All centres breed, hold and distribute wild type, transgenic and mutant animal lines but **the EXRC supports the needs of the international *Xenopus* community in three unique ways:**

(i) it is the world's sole centre for the collection, characterization and distribution of quality-assured molecular resources relating to *Xenopus* research;
(ii) it provides on-site service and training for one-off projects to non-*Xenopus* researchers;
(iii) it offers a 'research hotel' facility where visiting scientists can access the EXRC infrastructure and expertise and perform experiments.

Since 2014, and in response to priorities set by the *Xenopus* community (Xenopus White papers 2011, 2014), the EXRC has expanded its suite of molecular resources and services to include: testing, curation and distribution of *Xenopus*-specific antibodies; curation of a library of large sequences of frog genome; generation of novel transgenic *Xenopus* lines; and combination of gene editing and protein-tagging techniques to improve the detection of specific proteins (**G4, G5**). All EXRC resources are globally accessible via the EXRC website and Xenbase, the *Xenopus* bioinformatic knowledge base. Over the last three years, **the EXRC has supplied resources to over 180 laboratories in 25 different countries in the UK, Europe, North and South America and Asia**. Measured by growth in annual income from sales of frogs/products supplied at cost, demand has doubled over the last two years and continues to increase, indicating its significance within the research community.

Refinement and reduction of numbers of *Xenopus* used in research

The concentration of frogs at a single, central and closely monitored site has impacted positively on *Xenopus* welfare and has reduced the numbers of adult animals held globally. The [text removed for publication] confirms that '*the EXRC has made significant contributions to improvements in Xenopus welfare through reduction in animals used, refinement of research techniques and training for research personnel'* (**S1**). Over the last 3 years, EXRC staff have spent a total of 150 days visiting research laboratories in the UK, Europe and the US to provide training and expert advice on animal husbandry. The Executive Director, Institute of Molecular Biology, Mainz, Germany confirms that EXRC resources and frog husbandry advice *'…. constitute an irreplaceable platform for the international Xenopus community at large'* (**S2**). **Sperm freezing and streamlined oocyte preparation procedures developed at the EXRC have reduced the number of animals needed to provide the same volume of bioresources**. Additionally, transport as sperm or eggs eliminates stress on live animals and offers cost savings to researchers and funding bodies. Since the beginning of 2014, the EXRC has supplied 1,400 batches of oocytes and, since 2018, shipped 200 testes and 1,500 batches of frozen sperm to laboratories around the world. As a result, several laboratories have discontinued hosting local colonies of adult frogs and now obtain all of their *Xenopus* bioresources from the EXRC, thus reducing the total number of adult animals in captivity (**S3**). **The EXRC actively promotes the uptake of 3Rs techniques**. For example, the EXRC sperm freezing protocol (**R3**) was presented to UK and international audiences at the European Amphibian Club, Rennes (June 2017), the NC3Rs/Zoological Society of London Workshop on Amphibian Welfare (October 2017) and the *Xenopus* Genome Editing Workshop, Woods Hole (October 2019). A video on 'Using Frozen Sperm' is available on the EXRC website: https://xenopusresource.org. Between April and December 2019, EXRC staff visited key research centres in the UK (Universities of York, Cambridge, Aberdeen and UCL) and Europe (CNRS Paris, NHM Paris, DKFZ- Heidelberg, Munich Medical School, Barcelona Medical School, University of Ghent) to train researchers in the use of frozen sperm. Further visits and a workshop in Portsmouth planned for 2020 were curtailed by COVID-19.

The EXRC has driven the incorporation of '3Rs' into policy and practice

The EXRC has led on community initiatives to extend and embed the '3Rs' into national and international guidelines on the use of *Xenopus* in research. In 2019, and based on expert advice from the EXRC, the NC3Rs published a new checklist to assess the welfare standards in research proposals involving the use of *X. laevis or X. tropicalis* (**S4**). Adherence to this checklist is required to meet the expectations of all major UK public funders of *Xenopus* research conducted in the UK and overseas, in accordance with UK legislation, 'Responsibility in the Use of Animals in Bioscience Research'. The EXRC also acts as a "hub" for the *Xenopus* research community in the UK and Europe and lobbies to influence international policies on its behalf. Currently, the European Commission is undertaking a risk assessment to designate *Xenopus* as an Invasive Alien Species, based in part on concerns about transmission of amphibian diseases to native species. Such a designation would significantly restrict the use of *Xenopus* in research. In April 2019, Guille submitted a community response that confirmed the implementation of improved biosecurity measures at laboratory and breeding facilities in the UK and Europe (based on **R2** and led by the EXRC), and provided a robust evidence base for *Xenopus* as a key model for biomedical and environmental research, with significant socio-economic benefits (**S5**). A revised Commission proposal has been delayed by COVID-19 but is expected in summer 2021.

Improvements in the efficiency and competitiveness of the national and international *Xenopus* research base

National investment in *Xenopus* research resources has yielded an outstanding return in published new discoveries benefitting science, medicine and society. A PubMed search using the term "*Xenopus*" retrieves over 4,500 papers in the last five years: UKRI Gateway to Research identifies 17 active *Xenopus* projects totalling GBP3,900,000 (09/11/2020). The EXRC enhances the efficiency of research by *Xenopus* users' laboratories through:

(i) **acting as a centralised curator of frog lines and reagents**. The EXRC curates a full collection of reagents and animals from laboratories around the world: together with an efficient website and good administration, this enables the global science base to access *Xenopus* resources more efficiently, as well as reducing the time and effort spent by producer laboratories on sending out reagents. Since 2014, the EXRC has also 'rescued' valuable and expensive reagents generated by laboratories that are downsizing or when research leaders retire, including antibodies, large collections of plasmids, fosmid libraries and key *Xenopus* lines. Without these efforts, these reagents would be lost to the research community and their replacement would require significant time and resource.

(ii) **saving costs associated with keeping local *Xenopus* colonies.** The cost of keeping a *Xenopus* colony of 20 animals, with suitable technical support, is in the region of GBP25,000 p.a. compared to GBP4,500 to obtain equivalent bioresources from the EXRC. Dr Ian Mellor, whose research develops leads for new drugs for Alzheimer's disease, confirms '*when the EXRC made quality-assured oocytes available, we stopped keeping our own Xenopus colonies altogether.* (This) *…was not only more cost effective than housing our own animals, but also provided greater experimental efficiency by removing a step in the protocol'* (**S3**).

(iii) **providing quality assured frog lines and molecular reagents**. This saves both time and money for research laboratories and for funders of research. Prof Jeremy Green at the Centre for Craniofacial & Regenerative Biology, KCL, sources wild type frogs, frozen sperm and shipped testes from the EXRC for a project, funded by the NC3Rs, that promises to halve the number of *Xenopus* used for fertilisation and embryological study worldwide. He confirms '*The validity of our results depends absolutely on the consistency of the animals and of the sperm or testes… '* and *'having the frozen sperm protocol and shipped testes (not available elsewhere) has had a huge impact on my ability to deliver worthwhile results'* (**S6**).

(iv) **offering facilities, expertise and training to visiting researchers**: between 2018 and 2020, over 60 scientists attended the EXRC research hotel for a total of 190 days. In this way, the EXRC **delivers highly skilled scientists who are knowledgeable about the 3Rs** and, for researchers who lack the facilities or knowledge at their home institution, **the ability to access high-quality, specialised resources and to generate pilot data that is vital to their research careers**. Data generated at the EXRC by Dr James Cobley, an early career researcher, has underpinned key publications and successful grant applications. He says *'my work is only possible because the EXRC exists. It is, therefore, difficult to overstate the profound impact EXRC has had on my research programme'* (**S7**).

Enhancing the capability and competitiveness of international CROs

Xenopus development is highly sensitive to environmental changes. In June 2018, concerns over the safety of chemicals with endocrine-disrupting properties, found in pesticides, pharmaceuticals and household products, prompted relevant European authorities to issue new regulatory guidance. This included two *Xenopus*-based tests as mandatory components for registration of pesticides and biocides in Europe. Consequently, contract research organisations (CROs), which regularly undertake regulatory testing, had to rapidly extend their testing capacity and capabilities in order to continue to provide a full-service portfolio in environmental toxicology. Since July 2019, the EXRC has supplied *Xenopus* embryos to three globally-significant CROs: Covance, Charles River Laboratories (CRL) and ibacon. The Study Director Environmental Sciences, CRL, confirms '*This high quality supply is essential for us and our clients. Alternative quality sources of Xenopus are not available and to keep our own frogs would be too time consuming and costly. Our ability to source embryos from the EXRC has enabled CRL to meet demand from the market, increase our testing capacity and helps to create a safe environment'* (**S8**).

Transforming the speed and accuracy of diagnosis of rare genetic diseases (RGDs)

There are over 6,000 known RGDs and 7% of the UK population is affected by an RGD, mainly as children. Despite transformational advances in mapping genome sequences, there are critical difficulties in linking a genetic change observed in a patient to their disease with sufficient certainty to allow clinical intervention. Delays in diagnosis are critical: more than half of children with an RGD will die before their 5th birthday (Blencowe et al, 2018), whilst 5-year survival rates rise to 80% upon diagnosis, and the patient's family suffers, with 83% needing support for their mental health. '*Research by the team at the EXRC using the Xenopus model has clarified variants of unknown significance found in genomic clinical testing and has enabled clinicians to describe two new syndromes by proving that variants found in two genes that were never associated with a human disorder were, in fact, causal. This insight is critical for clinicians to be able to develop targeted interventions for treatment or management*' (**S9**). Additionally, the speed with which results from the *Xenopus* studies are available has significant impacts on the speed of diagnosis and offers the potential for screening and prenatal diagnosis. '*This impacts patients through changes in clinical management, facilitating prenatal diagnosis, allowing relevant health screening and family cascade testing, as well as ending the diagnostic odyssey that many families go through*' (**S9**).

5. Sources to corroborate the impact

S1. Letter from [text removed for publication] (01/12/2020)

S2. Letter from Executive Director, IMB, DKFZ-Heidelberg, Germany confirming the value of the EXRC to the international *Xenopus* community (28/10/2020)

S3. Letter from Dr Ian Mellor, University of Nottingham, confirming cost and experimental efficiencies to research programmes (06/11/2020)

S4. NC3Rs publication: Additional questions on the use of *Xenopus laevis & X. tropicalis* overseas (2019) and confirmation of EXRC contribution: Discussing the future of amphibian research, NC3Rs article (Nov 2018)

S5. Letter to the European Commission DG Environment submitted by Guille, on behalf of the UK and European *Xenopus* community, in response to Commission proposals to designate *Xenopus* as an Invasive Alien Species (22/04/2019)

S6. Letter from Professor Jeremy Green, King's College London corroborating the quality and value of EXRC resources to research programmes (20/10/2020)

S7. Letter from Dr James Cobley, Division of Biomedical Sciences, University of the Highlands and Islands confirming the value of the EXRC his research (20/10/2020)

S8. Letter from Study Director Environmental Sciences, Charles River Laboratories, Netherlands, corroborating benefits to capability and commerce (06/11/2020)

S9. Letter from NIHR Research Professor Genomic Medicine (Faculty of Medicine, University of Southampton) and Honorary Consultant in Clinical Genetics (Wessex Clinical Genetics, University Hospital Southampton NHS Foundation Trust) confirming the clinical impacts of EXRC research on the treatment or management of rare genetic diseases (11/02/2021).