Institution: University of Chester		
Unit of Assessment: UoA5 Biological Sciences		
Title of case study: Novel tools for animal welfare: maximising robustness and effectiveness of stress assessment in mammals and amphibians under human care.		
Period when the underpinning research was undertaken:		
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:**
Tessa Smith	Professor	2002 – ongoing
Charlotte Hosie	Dr, Senior Lecturer	1994 – ongoing
Robert Coleman	Dr, Senior Lecturer	2009 – ongoing
Christina Stanley	Dr, Senior University Teaching Fellow	2015 – ongoing
Period when the claimed impact occurred: 2010 – 2020		
Is this case study continued from a case study submitted in 2014? N		

1. Summary of the impact (indicative maximum 100 words)

Animal welfare assessment is a rapidly growing field, demanding tools that are highly sensitive and rigorously robust, to ensure best possible care of animals. We have developed important novel tools, combining non-invasive endocrinology, behavioural observation, and latterly, Social Network Analysis (SNA), to maximise highly reliable (stress free) welfare assessment. Nationally and internationally, these tools have enabled significant improvement of individual animal welfare in three species of captive mammals, improving conservation breeding programmes or laboratory research use. Our work also has enabled major welfare improvement for the main laboratory amphibian model, *Xenopus laevis*. Biological material from this species has been in wide use globally (particularly for medical research into human disease) over decades, but the animals' welfare needs have been little considered, until now.

2. Underpinning research (indicative maximum 500 words)

High quality captive animal welfare research fundamentally depends on the development of precise, accurate and highly reliable tools to assess welfare parameters, particularly stress assessment. The group's primary aim has been development of key tools to **non-invasively** assess welfare of captive animals, to improve conservation management/breeding (in internationally important zoos) or laboratory research-use welfare.

Social relationships and welfare assessment

Smith and **Coleman** have comprehensively validated novel biochemical assays that precisely quantify minute levels of glucocorticoid stress hormones: cortisol/corticosterone. Importantly, the work focuses on working with media collected non-invasively, minimising stress caused by sample collection as far as is possible. This has led to high-quality papers on social relationships and welfare status in mammals, particularly primates **[R1; R2; R3]**. **Smith** and **Coleman**, with Jersey Zoo (JZ), have detailed the endocrine status and social relationships of the captive pied tamarin group, to better understand the health, welfare, and reproductive success of this critically endangered species **[R3]**. **Smith** (since 2009) with University of Stirling, has characterised social relationships and impacts of rearing conditions on common marmosets in research colonies **[R1, R2]**, valuably demonstrating positive welfare for hand-reared animals. Reflecting her esteem, **Smith** has been invited to coordinate various conferences/workshops, notably for the Primate Society of Great Britain, and to organise and chair a keynote workshop on endocrine assessment of welfare in wild animals: Society for Experimental Biology (SEB) International meeting 2018.

Since 2015, **Stanley**'s innovative use of Social Network Analysis (SNA), to determine and quantify social relationships in mammals from direct behavioural observations, has enabled an important focus on individuals' social experiences. Work with JZ and their Livingstone's fruit bats, has recently demonstrated complex social relationships **[R6]** with important implications for individual welfare assessment and enhancing breeding programme success.

Amphibian welfare: breaking new ground

Smith's collaboration, with **Hosie**'s work on amphibian behavioural ecology has, since 2010, highlighted the much-neglected area of amphibian welfare. Pilot work (**Smith, Coleman**) developed an entirely novel biochemical assay for amphibian corticosterone (stress hormone), sampling, non-invasively, the water surrounding a frog; this assessment was previously lethal or highly invasive/stressful (from blood). This work led to funding by National Centre for Refinement, Reduction & Replacement [NC3Rs], £318,366 over 2013-16, the first major funding for amphibian welfare), and developed novel, non-invasive endocrinological and behavioural tools to refine (by NC3Rs definition) husbandry for the globally important amphibian model *Xenopus laevis*. Our work (**Smith, Hosie, Coleman**, with PDR Holmes and Research Technician Emmans) showed positive impacts on welfare through husbandry changes. Provision of dark background colour for tanks reduces stress **[R4]**, and transportation of frogs is acutely stressful with longer-term impact on settling into new environments **[R5]**. This work has been used to make dramatic changes in the way Xenopus frogs are distributed internationally. A further three-year project, in collaboration with Kings College London (Professor Jeremy Green, lead PI; **Smith, Hosie** co-PIs; NC3Rs funding) examines whether double ovulation protocols for female *Xenopus laevis* are stressful. Preliminary work suggests this could drastically reduce the number of females required in laboratories.

3. References to the research (indicative maximum of six references)

[R1] Ash, H., **Smith**, T. E., Knight, S., & Buchanan-Smith, H. M. (2018). Measuring physiological stress in the common marmoset (*Callithrix jacchus*): Validation of a salivary cortisol collection and assay technique. *Physiology & Behavior*, 185, 14–22. https://doi.org/10.1016/j.physbeh.2017.12.01

[R2] Hayley Ash, Tessa E. **Smith**, Hannah M. Buchanan-Smith (2020). The long-term impact of infant rearing background on the behavioural and physiological stress response of adult common marmosets (Callithrix jacchus). *Applied Animal Behaviour Science*, Volume 234, 2021. 105169, doi.org/10.1016/j.applanim.2020.105169.

[R3] Price, E., **Coleman,** R., Ahsmann, J., Glendewar, G., Hunt, J., **Smith**, T., & Wormell, D. (2019). Individual, social, and environmental factors affecting salivary and fecal cortisol levels in captive pied tamarins (Saguinus bicolor). *American Journal of Primatology*. doi.org/10.1002/ajp.23033

- Featured Article in the Amer J. of Primatol. in August 2019; promoted by American Society for Primatologists & Wiley & and made freely accessible for three months.
- In the top 10% most downloaded publications Amer J. of Primatol (between January 2018 and December 2019). To quote the editor: 'Your research generated immediate impact and helped to raise the visibility of American Journal of Primatology.'

[R4] Holmes, A. M., Emmans, C. J., Jones, N., **Coleman,** R., **Smith**,T. E., & **Hosie**, C. A. (2016). Impact of tank background on the welfare of the African clawed frog, Xenopus laevis (Daudin). *Applied Animal Behaviour Science*, 185, 131-136. doi.org/10.1016/j.applanim.2016.09.005

[R5] Holmes, A. M., Emmans, C. J., **Coleman,** R., **Smith**,T. E., & **Hosie**, C. A. (2018). Effects of transportation, transport medium and re-housing on Xenopus laevis (Daudin). *General and Comparative Endocrinology*, 266, 21-28. doi.org/10.1016/j.ygcen.2018.03.015

- Resulted in award of £309,101 (2019-22) from NC3Rs for 3-year research programme **Smith, Hosie** with J. Green (King's College London).
- Chapter invite **(Hosie):** 'Behavioral Biology of Amphibians' (Hosie & Smith); in Behavioral Biology of Laboratory Animals eds Coleman, K. & Schapiro S. out in 2021 US, then UK.

[R6] Welch MJ, **Smith** T E, **Hosie** C A, Wormell D, Price E & **Stanley**, C R. (2020). Social Experience of Captive Livingstone's Fruit Bats (*Pteropus livingstonii*). *Animals*, 10(8), 1321; doi.org/10.3390/ani10081321

- **Stanley** invited author, special Issue: Evidence-Based Practice Zoo Animal Management

4. Details of the impact (indicative maximum 750 words)

Our non-invasive tools for assessing stress in a range of species have had wide and multiple impacts. Some arise as a direct result of our work, others through dissemination and stimulation of further welfare-targeted work by key stakeholders in zoos and laboratory animal use.

Animal Welfare: captive primate impacts

During 2018, 110 experimental procedures were conducted on 90 marmosets born just in the UK –8% increase on preceding year (Annual Statistics of Scientific Procedures on Living Animals Great Britain 2018). Professor Colleen Schaffner, primatologist for 32 years, including nine years working with callitrichids including non-invasive measures of cortisol confirms: "In light of the large numbers of marmosets maintained in captivity globally and the increasing demand for marmosets as models for neuroscience research, the impact of studies **[R1, R2]** is likely to be significant. For example, there were 1900 marmosets in research facilities in the USA in 2018 and the number is growing". The work by Smith (et al) developed and validated a novel method for quantifying cortisol in the saliva of the marmoset monkey **[R1]**. This was then used **[R2]** to directly evaluate the behavioural and physiological impact of supplementary feeding of marmoset monkeys born into triplet litters. Schaffner says **[S1]**: "Triplet litters, although not the norm for marmosets in the wild, are increasingly common in captive colonies. Since parents can rarely raise all three monkeys, human intervention through supplementary feeding of one monkey, is required. **R2** revealed minimal behavioural and physiological impact of this feeding, suggesting the rearing practice is a viable option to improve infant survival from large litters." **Smith**'s work has had immediate positive welfare impact on the marmosets participating directly in this research (published last year), but also, Schaffner advises: "can underpin management protocols for rearing triplet litters, benefiting animal care staff and the finances of research institutions in terms of increased animal survival. The work has important implications for the welfare of the marmosets" **[S1]**.

Head of Research at JZ (Dr Eluned Price) identifies **R3** as the first research to comprehensively explore the factors modifying stress in another callitrichid, the pied tamarin. JZ houses around the largest captive population (18%) of this species globally, 23% of European population. **R3** has generated incipient impact by being shared across international partners, especially those in the species' native Brazil. Dr Price: "In light of the documented suppressive influence of stress on reproductive function, understanding the stress response in pied tamarins is crucial to maximise the breeding success of this endangered primate (and other endangered tamarins, [**S2]**)". As a direct result of this research, JZ can now: "identify factors associated with increased stress in captive pied tamarins. Information obtained from this research now guides management decisions in the captive population, to maximise their welfare and minimise stress **[S2]**." For example, they identify that the unexpected result that tamarins housed on show to the public had lower cortisol levels than those not housed on show, has led to changes in housing to reduce stress levels for this important group. Our work has had direct positive welfare impacts on these animals, individually, and the vital breeding programme of which they are a key part. It has also prompted future work outside of UoA5: a PhD examining how different potential sources of stress, acoustic versus visual stimuli, affect the tamarins **[S2]**. JZ also say: "the results of these studies are relevant not only to captive populations, but to the wild population of this species, which lives in a landscape in the Brazilian Amazon severely affected by human disturbance **[S2].**" This further underpins the key role of JZ's conservation breeding programme, and the importance of the improved welfare in these animals that has been prompted by our group's research.

Animal welfare: captive fruit bats

The SNA research on captive Livingstone's fruit bats housed at JZ, 80% of the worldwide captive population, has been ground-breaking. Head of Research (Dr Eluned Price) and Head Keeper (Dr Dominic Wormell) identify **[S3]** that this is the first research project, globally, to comprehensively explore the social experiences of individual Livingstone's fruit bats. They can now identify closely bonded individuals based on their spatial associations, and predict their social role in the group based on sex and dominance level. This information is used "when removing individuals from the group for veterinary purposes, aiming to remove individuals along with their closely bonded 'partners' where possible" **[S3]**. This study has already (out 2021) had a significant positive impact on the individual welfare, through captive management improvements, of this large population of

Livingstone's fruit bats. Future husbandry management decisions, including the transfer of individuals to other breeding colonies, can now be strongly evidence-based, and so minimise stressful impacts on both individuals, and the breeding group as a whole.

Amphibian Welfare: refining captive *Xenopus laevis* welfare and reducing their use

We were invited to present work from our 2013 NC3Rs funded project **[R4, R5]** at SEB's 2016 Animal Symposium (co-hosted, NC3Rs & ASAB): 'Improving Experimental Approaches in Animal Biology: Implementing the 3Rs', where **Hosie** won the ASAB prize for best oral communication. This was for demonstrating improvements to *Xenopus* husbandry (plus an additional ASAB poster prize presented by Holmes et al). Our work was main feature of the SEB newsletter autumn 2016, and prompted the NC3Rs to invite **Hosie** to speak at the next Animal Technicians' Symposium, a joint NC3Rs/Institute of Animal Technology event (2016) with 123 delegates from 36 UK research institutions. The was talk was recorded and made available as an NC3Rs training aid **[S6]**. It has been viewed 28 times (password protected), reflecting the interest of the small but important number of UK technicians involved in *Xenopus* care. Some of our work **[R4]** was featured as an NC3Rs blog (2016) to highlight improvements Xenopus users might make in their animal colonies **[S7]**. It has been viewed 660 times, mainly from UK, US, and Canada. We have made internal policy changes in management of our frogs at Chester. We know now that female's stress levels decrease by 29% when housed on dark backgrounds, so our tanks are wrapped in black fabric. Anecdotal chats at conferences also indicate positive findings for animals on black backgrounds: females appear less stressed, and may produce more eggs. More work on this will follow.

Our work showed, for the first time, that transportation of live *Xenopus laevis* frogs elevates their stress levels, acutely, but has lasting impacts: it takes in excess of a week for behaviour and stress physiology to return to normal. This is highly relevant to distributors of live animals as any reduction in transportation could have major impact on amphibian welfare. Matthew Guille, Professor of Developmental Genetics at Portsmouth leads the European *Xenopus* Resource Centre (EXRC) funded by the Wellcome Trust, BBSRC, MRC, and NC3Rs. It designs, creates, collects, curates, and then distributes research resources in support of biomedical and environmental scientists using *Xenopus* models. Professor Guille identifies the impact of our work on his operations **[S4]**. He saw our SEB (2016) talk and identifies: "this struck me immediately as highly relevant to the work of the EXRC since we distribute >1500 live research animals to laboratories as far away as South America and Japan annually **[S4]**". Our data also showed a significantly higher stress hormone elevation for male animals compared with females. This negatively impacts on their welfare and ability to assume reliable laboratory roles, in this case breeding to produce embryos for research. Professor Guille continues: "EXRC is committed to providing high quality research resources and minimising animals' negative experience wherever possible. That transport is stressful is a finding that carries great impact. Males are generally transported to provide sperm for *in vitro* fertilisation of females' eggs. Hosie and Smith's finding suggested that supplying sperm to laboratories, rather than whole male animals, would enable an enormous reduction in the number of animals experiencing stress through transportation. This change would also enable more cost-effective transport of male gametes, saving research funds. EXRC submitted a bid on the basis of this finding, funded from 2017: NC3Rs https://www.nc3rs.org.uk/news/reducing-use-male-**xenopus**-frogs" **[S4]**.

Our work has had great significance, globally, in a dramatic reduction in global use. As Professor Guille highlights: "EXRC's transportation of frogs has been altered as a result of this work; the number of male frogs sent has dropped precipitously. Over the last 3 years more than 1,500 batches of frozen sperm and 100 chilled testes have been supplied to researchers around the world and this method of sending male gametes has also been adopted by the US stock centre, the NXR. Some laboratories have now stopped keeping adult male frogs altogether, resulting in welfare and cost savings" **[S4]**. Following this, there have been further changes with far-reaching animal welfare impacts: "the realisation that animal research resources could be moved reliably from the resource centres has had further, knock on effects. Some groups using embryos older than one day have now stopped keeping adults altogether and simply get embryos sent. Since frogs produce so many offspring, the eggs from one mating can often be shared between multiple labs, reducing the number of frogs used" **[S4]**. Professor Guille identifies: "Hosie and Smith's

original finding was the start of a transformational change in the way the resource centres are used by the community. This has reduced and refined the use of *Xenopus* in research" **[S4]**.

We have discussed our work widely at over 30 conferences (2013-19) including UK Xenopus Meeting x3; International Universities Federation for Animal Welfare (UFAW) x2; NC3Rs/BBSRC joint meeting; SEB x3; ASAB x2; Amphibian Conservation Research Symposium x3. Professor Guille identified after our 2016 talk: "it was clear… that their work could be used to raise the profile of amphibian welfare in the *Xenopus* user community. As joint organiser, I invited them to present their work at the 16th International Xenopus Conference – hosted by Xenbase, where it enabled discussions with users worldwide about *Xenopus* welfare and husbandry."

Our cumulative work in this area also resulted in an invitation (one of 6) to present the NC3Rs funded work, and additional wild amphibian research, at the first ever International Amphibian Welfare Symposium, organisers: NC3Rs & ZSL, October 2017. Discussions here prompted a joint bid from Professor Jeremy Green, with **Smith** and **Hosie**. Prof Green comments: "preliminary work in my group suggested the two protocols (double and single ovulations from females) elicited similar numbers of eggs per ovulation with no obvious detriment to the animals, despite more frequent ovulation. If females can be stimulated to ovulate twice as often (without additional stress) then the required number of eggs can be produced with half the number of female frogs. This could be a dramatic saving in terms of use of animals ('reduction' as defined by NC3Rs) and cost to laboratories **[S5]**. We applied for collaborative funding to test the Green protocol using our stress assay. The bid was successfully funded under the title Assessment of double ovulation to halve Xenopus laevis use for eggs (Jan 2019-Dec 2022) and was given the highest possible ratings by the review panels and supported by world-leading *Xenopus* laboratories. After some initial delays, including for COVID shutdowns, work is now underway, with postdoctoral researchers now in place at KCL and University of Chester. Prof Green identifies: "results from our studies would certainly lead to uptake by the community of researchers worldwide who use Xenopus species for obtaining eggs. Xenopus is a lab species that has been a pioneer in a very large range of discoveries, including Nobel prize-winning work, so our collaborative work promising a significant improvement in the way it is used in laboratories in future must count as a significant impact" **[S5]**. Overall, this work has made great positive impact on the welfare and use of *Xenopus laevis* in research. As a long-lived species (in captivity, up to 10-15 years), any improvement in individual welfare and wellbeing matters immediately, and adds up considerably over time, leading to improved life expectancy and fewer animals in laboratories. Our identification of the differential impacts of stress for males and females, and where this can be minimised, has also enabled changes that have led, and should further lead, to dramatic reduction in the worldwide use of this species.

Our combined work (Smith, Hosie, Stanley, Coleman) developing non-invasive tools for assessing stress, has clearly evidenced, important welfare impacts in a range of animal species in different contexts. These impacts have wide reach already, and will continue to develop further in the future.

5. Sources to corroborate the impact (indicative maximum of 10 references)

[S1] Statement letter, Professor & Program Chair of Psychology Director, School of Humanities & Social Sciences Adams State University.
[S2] Statement letter Durrell Wildlife Conservation Trust, Jersey Zoo
[S3] Statement letter Durrell Wildlife Conservation Trust, Jersey Zoo
[S4] Statement letter, European Xenopus Resource Centre, Portsmouth.
[S5] Statement letter; pro VC for International Research; Kings College London
[S6] 2016 NC3Rs/IAT Animal Technicians' Symposium presentationsNC3Rs/IAT
2016 NC3Rs/IAT Animal Technicians' Symposium presentations | NC3Rs
[S7] https://www.nc3rs.org.uk/.../black-or-white-effect-tank-background-welfare- laboratory-frogs-xenopus-laevis