ASSG32: Tadpoles as biomonitoring tools to assess Endocrine Disruption in Polluted Ponds

Catherine Whatley

Ph.D. student at the University of the West of Scotland

Corresponding email: catherine.whatley@uws.ac.uk

Background:

When last assessed in 2004, 32% of amphibian species were reported to be threatened with extinction and at least 43% were in decline, faster than any other vertebrate group (Stuart *et al.* 2004). Amphibians also act as important ecosystem indicators (Waddle, 2006). Aside from habitat loss, pollution is the most important threat impacting amphibians (Diaz *et al.* 2019). Chemical pollutants have a range of impacts on amphibians and other aquatic biota, including those that alter hormone functionality. Such pollutants are termed Endocrine Disrupting Chemicals (EDCs), and are defined as: chemical substances that can alter the function of the endocrine system by interfering with hormone receptors, hormone synthesis, or conversion of hormones, and can cause adverse health effects (WHO, 2002). Although pollutant profiles in small freshwater bodies where amphibians typically breed are not well known, due to their small size and thus low dilution ability they are likely to contain high concentrations of chemicals; many of which are known or suspected EDCs (e.g. pesticides, pharmaceuticals: Pinheiro *et al.* 2021). As many amphibians are entirely aquatic during larval stages, there is high potential for chemical exposure.

Biomarkers are an important component for field monitoring for the effects of chemicals (Lemartire *et al.* 2021). Due to their small size, high density, and tendency to complete their development within a small water body, larval forms of amphibians are excellent models for assessing exposure effects of these chemicals. In particular, the detection of EDCs that can disrupt developmental processes concerning sexual differentiation and development, which are highly dependent on hormonal activity (Woodley*, 2015*). Apart from vitellogenin induction as a biomarker of estrogenic activity, validation of biomarkers for detecting endocrine disrupting activity for reproductive modalities are lacking in amphibians (biochemical biomarkers review: Venturino and D'Angelo, 2005; reproductive physiology review: Orton and Tyler, 2015). As part of my Ph.D. programme, which has the overall aim of developing new biomarkers for the detection of EDCs impacting the reproductive system in wild common frogs (*Rana temporaria*), I investigated gene expression profiles in tadpole gonads (real-time qPCR: *dmrt1, amh, foxl2, cyp19, cyp17*) that have previously been shown to have importance in sexual differentiation and determination pathways in the model amphibian species, *Xenopus tropicalis* (Orton *et al.* 2018). Evidence for alterations to these pathways could have impacts on apical endpoints related to reproductive health (impact biomarker) and/or indicate for the presence of EDCs (exposure biomarker).

Purpose:

Although real-time qPCR is a standard method for the analysis of gene expression, this visit to Exeter University was vital to utilise their expertise in working with extremely low quantities of tissue (*R. temporaria* tadpole gonads are the size of a full stop). This presents technical challenges for the extraction and purification of sufficient high quality RNA. The University of Exeter is one of the leading institutes in the UK for the analysis of gene expression, with highly specialised equipment as well as with researchers with the experience of successfully isolating RNA from single cells, which I have had the opportunity to learn.

Results:

Although these data were collected successfully, analysis of the results is still ongoing. The gene expression profiles from these tadpole gonads will be combined with histological data from the same gonads in order to determine if these changes in gene expression profiles have any effect on morphological endpoints such as egg count within ovaries, or any evidence of intersex markers. This research visit allowed me the valuable opportunity to experience working in a new laboratory and research environment, and allowed me to network with other researchers in my field. I hope that the outcomes of this research in the context of the overall Ph.D. project will result in publication in a high ranking journal, as well as in-person dissemination events (such as National/International Conferences). I would like to thank MASTS for awarding me this travel grant, without which the undertaking of this work would not be possible.

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