1. PUBLISHABLE SUMMARY

Emerging infectious diseases (EIDs) are increasing worldwide, and have been implicated in population declines and species extinctions (e.g. *Mycoplasma gallisepticum* in house finches, and Chytridiomycosis in amphibians). We know that diseases can cause major changes to the genetic composition of host populations, and in turn that the genetic composition of host populations can influence the impact of disease. We also know that diseases can lead to changes in mate choice behaviour, which will also alter the population structure, again influencing the impact of disease - yet this has as yet received little attention.

Evaluating the ways in which diseases change the genetic composition of host populations and the impact that this has on susceptibility to disease remains a challenge, particularly in wild populations, yet this is critical for understanding the evolutionary implications of disease, or for modelling disease spread. One aspect that hampers advancement is that there are relatively few cases of diseases in wild host species where the host and pathogen can be both examined in the wild and manipulated in the laboratory. This project involved using transcriptomics (the study of which genes are read and translated into proteins) to investigate the interactions between genetics, behaviour, and disease in a vertebrate host-pathogen system that can be examined both in the wild and in the laboratory – Common frogs (*Rana temporaria*) with *Ranavirus* infection. This work is of direct importance for biodiversity and conservation as it addresses amphibian declines caused by an EU notifiable disease.

This project relied on a complicated field sampling logistics, and the participation of citizen scientists. Field sites (private UK garden ponds) which were either positive or negative for *Ranavirus* infection were identified. Experimental tanks were established at each field site. The pond owners (citizen scientists) were asked to wait for their frogs to begin mating, then place pairs of mating frogs into individual tanks. Once the frogs spawned, samples were taken from the parents and the egg mass. This approach allowed mate choice to occur freely and naturally, yet also allowed us to sample the parents and offspring. All adult frogs and the majority of the spawn were then returned to the wild.

This unusual and ambitious approach yielded samples for a number of different studies. We showed that populations with a history of *Ranavirus* infection had similar survival rates to experimental infection, regardless of dose, indicating a strong immune response. Populations with no history of disease responded differently – those exposed to the highest *Ranavirus* dose had the lowest survival rate, and those exposed to a control dose had the highest survival rate. The survival rate of control animals (those that remained uninfected) from both *Ranavirus* positive and negative populations were comparable. This indicates that populations which are naïve to the virus are less able to mount an effective immune response. Clear differences were also detected between clutches, with survival rates of some clutches far exceeding that of others. It is probable that the different responses to infection from frogs from infected and uninfected sites, and between individual clutches, are due to genetic differences.

A further study showed that *Ranavirus* infection causes changes to the way genes are read and translated into proteins (gene expression). We found strong and significant up-regulation (i.e. the genes are used to produce more proteins than expected) of a gene involved in the adaptive (in other words 'learned') immune response (AP4S1) only four days post-exposure to either *Ranavirus* or the pathogen that causes Chytridiomycosis (a fungal pathogen responsible for species extinctions). These data provide a valuable genomic resource for the amphibians, contribute insight into gene expression changes under pathogen infection, and suggest potentially interesting genes for future host-pathogen research. The results have been published open access in PLoS ONE. The sequencing data is publicly available via EMBL-EBI Array Express (E-MTAB-3632).

It is clear that the genetics of an individual or population of frogs is not the only aspect influencing susceptibility to *Ranavirus*. Using a long term citizen science dataset of frog mortality

reports from the UK charity Froglife, we were able to show that both anthropogenic and ecological factors also contribute to susceptibility. Whilst controlling for the geographic distribution of mortality events, disease prevalence increases with increasing frog population density, presence of fish and wild newts, increasing pond depth and the use of garden chemicals. The presence of an alternative host (the Common toad) reduces prevalence, potentially indicating a dilution effect. Ranavirosis occurrence is associated with the presence of toads, an urban setting and the use of fish care products, providing insight into the causes of emergence of disease. Links between occurrence, prevalence, pond characteristics and garden management practices provides useful management implications for reducing the impacts of *Ranavirus* in the wild. The results have been published open access in PLoS ONE. The data is publicly available via Dryad (http://datadryad.org/review?doi=doi:10.5061/dryad.v3h8h).

This research has already yielded results that are of use for disease biology and conservation. Understanding the genomic level responses to this pathogen will contribute to future research into how immune systems fight disease. An unexpected outcome is that we have shown that it is possible to reduce the impact of *Ranavirus* through simple changes to garden management. Avoiding the use of garden chemicals and the release of exotic pets like goldfish into ponds is something that anyone with a garden pond can do. Extensive media coverage of this work has meant that a wide audience has been reached, and it is likely that real impact has already been achieved.

The project is now continuing as a PhD project run jointly between the University of Exeter, the Institute of Zoology, and FoAM Kernow. The samples taken from the parent frogs from the original field work are currently being used to sequence an immune gene (the Major Histocompatability Complex) – this will allow us to see if parental mate choice is influenced by their immune genes, and to see whether this affected the susceptibility of their offspring to infection. In addition, sequencing data has been completed to look at the whole transcriptome s of the parent frogs. This will allow us to understand more about the response to infection in the wild.

There is a project blog and media coverage of the project to date can be listened to on BBC Radio 4 Inside Science and read about in The Independent, The Times, The Telegraph, The Daily Mail, the Falmouth Packet, and West Country. The work was also featured on BBC One News (Spotlight 6/3/14), and BBC Radio Cornwall.