Interview with Dr Matthew Fisher (Imperial University, London) about his research on the fungus *Batrachochytrium dendrobatidis* that causes the often lethal disease, *Chytridiomycosis* in amphibians.

Q. Why are you using frogs for your research?

00.03 We work on a disease, it's a fungus that affects frogs and this disease has been found out to be a major contributor to the global decline in amphibians that we are unfortunately currently seeing.
00:21 the fungus, I'm going to say the name. It's called *Batrachochytrium dendrobatidis* but for obvious reasons we call it BD because it is a bit of a mouthful. Has been spreading around the world and these frogs that you see around you are affected by this fungus.
00.37 So *Chytridiomycosis* is the disease that the infection causes in amphibians. So if you imagine this fungus, it's an aquatic fungus and it swims through the water, it looks like a tiny tadpole and it's actually smelling the frog's skin and when its finds a frog this zoospore as we call it, will burrow in through the frog skin and then it will just disseminate through the skin and causes the infection.
01:07 Frogs depend on healthy skin function for their survival, so they take in water through their skin, they take in water through their skin and so anything that disrupts their skin function is going to impact on the health of the amphibian and what we find is that they lose control of their general homeostasis, their kind of body balance. Actually what eventually kills the frog is a heart attack but that is caused by it's disrupted skin function.

Q. 01:39 Why is *Chytridiomycosis*is an important disease?

01:43 In the 1970's, scientists, herpetologists studied frogs around the world realised that frogs were going extinct at rates that were ten thousand time above background, so they said hey what's going on here? So the obvious first point of call was habitat destruction, with these rainforests being chopped down that the frogs lived in.
02.06 Turned out that this was not the case, these amphibians were being lost in pristine environments that were, had never been really touched by humans. To cut a very long story short this fungus, BD was discovered as being an infectious agent that was spreading, a bit like HIV-AIDS did in humans but in an amphibian populations around the world and not all species were impacted but a great many were and of those, at least 200 have been driven to extinction by this fungus.

Q. 02:40 Is this a recently arisen disease?

02:43 Is BD a recently arisen disease? It's a very good question that. The short answer is yes and no. So no, because amphibians are the oldest land dwelling vertebrates that there are, 400 million years old. They have likely been living with …….. all this time quite happily. But what has changed is that a virulent highly virulent lineage of BD emerged somewhere in the world we don't know exactly where and spread very rapidly around the world, probably in the 20th century to invade and kill all these amphibians populations and species.
03:26 So no *Chytrids* are very ancient but yes this is a new phenomenon.
03:33 Is it a single disease or is it caused by a group of related diseases?
03:37 So until 2014, we thought this was a single disease Bd. In fact we thought that it was a single lineage of the single disease which we call BdGPL, which is the Panzootic Lineage –

the bad guy which has been extirpating amphibians around the world. Then scientists working in the Netherlands discovered that the fire salamanders there were going extinct and that they discovered that there was another *Chytrid* which is subsequently been called *Batrachochytrium salamandrivorans* - the salamander killer. So that's now, there is now at least two *Chytrids* which kill amphibian species. There are likely to be more out there and this is what we are hunting at the moment.

Q. 04:23 How does the fungus causing *Chytridiomycosis*is spread?

04:31 How is Bd spread? We've been looking at this, we know that frogs in the trade, so people love to keep frogs and they are delightful pets, I have some at home. What we are finding is that in these amphibians which are taken out of tropical rain forests and traded around the world, quite a high proportion of these are infected with Bd and we have actually shown that some of these infected trade animals when released into environment have spread their infection into the environment. We believe that the amphibian trade is a very important vector of this infection around the world.
05.10 We have been looking very closely at the trade and asking the question, should we be allowed to trade these species which are known to be infected with a virulent pathogen that could wipe out precious species in the environment? What we believe should be done is that the trade should be stopped for certain species which present a very high risk, so for instance the United States has enormous amount of salamanders, it's major vertebrate bio-mass is salamander. They don't have this new *Chytrid* B.sal as we call it. Therefore if B.sal got from the Netherlands to the United States it would be a very bad thing.
05:54 So what the United States has done is they thrown up a trade barrier to 201 species of Salamander from south east Asia and from Europe in an effort to prevent that infection from coming in on traded animals. We believe this type of ban should be erected for other countries and other species until we know which species are carrying those virulent infections and what we can do about it. It's perfectly possible to breed amphibians in facilities like this and to have them certified as pathogen free and then to be bought as pets. So those are clean animals and that's good and none were taken from the rain forests. There are many ways to look at this, but yes we believe that controls in trade need to be instigated.

Q. 06.41 How does the fungus infect a frog or other amphibians and what does it do to the animal?

06.46 The zoospores that are produced by Bd swim through the water and when the strike the amphibian skin they deploy some very potent enzyme called proteases. These zoospore literally burn their way into the amphibian skin and they then become intracellular and go through subsequent reproductive cycles to colonise the skin of the amphibian and it's at that point that skin function is disrupted and that the animal will fall sick.

Q. 07.18 What can humans do to help frog populations recover from this outbreak and prevent further outbreak?

07:25 Certainly no amphibian that's ever been kept at home should ever be released into the wild because it may have picked up an infection in your house from other amphibian species you may keep, that you bought from say south east Asia. We need to stop these vectoring of these pathogens from our homes into the environment. We need to be looking at trade to make sure we don't transport new unnoticed infections around the world in our amphibian species and other species that we would like to keep as pets. We need to also ensure that the

amphibians themselves have appropriate habitats so that if they are uninfected populations out there which there are, that they are able to survive and potentially recolonise populations have gone extinct.

Q. 08:22 What have you found out about this disease?

08:26 We found a great many things out about this disease. We found that not all genotypes or forms of this fungus are equally lethal and that there is one particularly lethal form that is causing the majority of declines. So this means that we can use genetic techniques to figure out what exactly it is that this bad guy does to be quite as bad as it is and that might give us a clue to future avenues for treatment and prevention we could use. Certainly form using these animals here we have developed new forms of treatment that show, that prove that we cure the infection in these animals and that this gives us a technique that we could potentially cure infection in the wild and rescue species from extinction.

09:19 What treatment is that, how do you actually do it?

09:23 The treatment that we have developed here in the laboratory is an anti-fungal drug called Itraconazole, this is commonly used in humans and pets. We have found that if we very dilute concentrations of this anti-fungal with tadpoles or these metamorphs then they will clear infection. The *Chytrid* is thankfully very, very, susceptible to Itraconazole. So by finding the exact dose that we can use on an animal without harming it, we can then go into natural environments and try those doses on infected animals and then to clear them. So we have actually done that.
10:03 We are very excited about this anti-fungal treatment that we have developed, we asked the question will this work in nature? So I have a fantastic collaborator in Spain called Heine Borsch and he works on the island of Majorca where this fungus was unwittingly introduced to the island by a Zoo that released captive-ly bred animals to increase the size of natural populations but unfortunately those animals have picked up infection in their colony so the *Chytrid* was introduced to the island of Majorca.
10:37 Now we figured out which populations were affected by the fungus and it took us 6 years to do this but went in there year after year, I say we, Heine Borsh went there year after year, sometimes he had helicopters to lift these tadpoles out, sometimes it was just backpacks with water bottles with tadpoles in and air bubblers, it was an Herculean effort but once these tadpoles were in a captive environment, they then got the Itraconazole treatment. They were kept until we were sure they were clear of infection and then they were re-introduced back into the environment. So in association with this we also use some chemical disinfection in the environment, where we wash rocks with this compound called Virkon which cleared any residual stages that was of the - fungus that were clinging to the rocks and by doing this we eventually cleared the infection from the island of Majorca.
11.33 This is a massive coup because this is the first time that a wildlife disease has actually been combated and eradicated in nature. So this avenue can now potentially be rolled out to other populations and other species. So from that original proof of concept we are now moving to mainland Spain where there are larger populations and they are more connected than this isolated Island community and we are going to now try this treatment on a much wider set of populations to see if it has any utility in a more real world context.

Q. What species do you use? Please tell me about their lifecycle - and can you speed up the natural lifecycle in the lab?

12.10 So the frogs we use here, they are called midwife toads, they are not actually toads but that's their name. They look slightly toad-like, their scientific name is *Alytes obstetricans,* the ones that live on the island of Majorca are called *Alytes muletensis* – a sister species. This species is found widely across Europe. These midwife toads are the most susceptible species to the fungus. So we work in the high Pyrenees where all these animals come from and at these altitudes above 2000 metres we see very aggressive die-off of these *Alytes* in a number of populations and in a number of populations they now no longer exist because the fungus has killed them all.
13:01 So we have been going these population year after year and we have been looking at the epidemiology of the pathogen. We have been asking questions on how it kills the animals, how many animals it kills, are the populations declining or increasing and whether there is any potential mitigation that we can do in those environments.
13:22 The animals, they have these very large tadpoles which you will see in these tubs and these tadpoles are rather special in that they won't develop within 3 or 4 weeks like other amphibian tadpoles do, they can sit there for up to 5 years in the lake. So this enables them to go to very high altitudes where the season for development is very, very, short, only a few months is when these lakes are unfrozen. Certainly from the point of view of working in a laboratory, they are not a great species to work with because their life cycle can take incredibly long and some of these tadpoles here are 2 or 3 years old.

14:03 **You asked question, can we speed this life cycle up?** So far we have not been able to do this. We want to continue working with this species because this is the one that matters, this is the one that is being extirpated and exterminated by the fungus so we want to find ways of working with the species that are applicable to saving it in a natural environment.
14:26 You are right in saying that we should be looking at other species that we can use to do our research so that we don't need to go into the wild and use midwife toads such as this. We have had some success in infecting zebra fish embryos as an amphibian-like aquatic organism. That has a very rapid life cycle and you can do much larger experiments, so hopefully we are going to be able to increase the number of species we will be able to work with so that we can do more powerful experiments that will get us further faster towards finding out how we save these guys in nature.
15:02 This is a broad spectrum fungal infection that infects hundreds if not thousands of species around the world. So already scientists are using our Itraconazole treatment protocol in other species in Panama, in Costa Rica, in South Africa and they are having some success in at least being to create captive amphibian arks where they have clean animals without a fungal infection that are breeding, so that when we do come up with a mitigation approach that we can use in the environment then we actually have these captive species that we can then reintroduced to the wild. So at the moment this is the only hope we have for the rain forest of Central America where this disease has had a very aggressive impact.
15:54 Because this is a generalist fungal pathogen and it affects hundreds, if not thousands of species of amphibian, what works in this species has a high probability of working in another species. So approaches that we identify, we are looking at some vaccine approaches that will work in *Alytes obstetricans,* will hopefully be portable to something such as the Panamanian golden toad which is being extirpated wild like *Chytridiomycosisis*.

Q. Are your findings about Chytridiomycosis relevant to other fungal diseases - diseases of man or even plants for example?

16:28 The short answer is yes, what we have shown is that there is a broad global process underway where *fungi* which were previously scattered in isolated populations around the world are being increasingly spread through trade mechanisms, this is something that people who work on plants are seeing. There are number of aggressive fungal infections which affect crops and Bananas is a hot topic at the moment because the Cavendish banana is being struck down by a *Fusarium* species which is being traded around the world in bananas. This is going to cause a global decline in the banana and certainly that is something that people rely on for a large proportion of their carbohydrates.
17:21 There are many other plant pathogens similarly being spread around and the drugs that we use to treat these frogs are also the same class of drugs that people who work on plant fungal pathogens use to treat plants. There are many commonalities between the epidemiology of the system that we are working on with frogs and *Chytrid* compared to the system where people are working with plants and plant pathogenic fungi.
17:47 Fungi as a class of pathogens are a real problem with humans because they prey upon humans who are immunosuppressed. It's not a widely recognised fact, it's a fact nonetheless that the top 10 fungal pathogens kill more people than those killed by Malaria and Tuberculosis put together. This is a huge burden on public health and a number of these *fungi* are being spread around the world human to human transmission. So we have a merging *fungi* as well as *fungi* that affect people who are immunocompromised. We need to know more about classes of drugs that we can develop to attack this *fungi*. We need to know more about where these *fungai* are coming from and how they are being spread within the human population.
18:36 So the stories that we are seeing with these frogs have many parallels both in human populations but also plant populations. It is a very broad and global problem.

Q. 18:48 Is the spread of fungi causing by fungi recombination? Are you getting new varieties or species?

18:56 Absolutely. One of the genetic questions that we have been working on with these fungal pathogens is when you have say a lineage that comes from South America, contacts a lineage from Asia, do they actually mix their genomes together through the process of recombination to create a hybrid. The answer to that is yes the do. This is being picked up recently on the Brazilian Atlantic forests where BDGPL, the bad guy has come into contact with BD Brazil which is a Brazilian *Chytrid* that infects the frogs there but it seems to be a fairly innocuous infection and hybrids have been discovered of those two lineages. Now we so far don't know whether the hybrids are more or less aggressive than either of the parental genotype.
19.47 What we do know with hydrolysation is that it throws up big diversity in progeny daughters and sons from the original mating event and some of those may have pretty wacky characteristics that could make them more virulent and would thus spore an ongoing epizootic in the amphibian populations. So recombination is a very dangerous mechanism especially when you package genomes together from other sides of the planet.
20.21 As you can see from this animal room here, we have tadpoles in tubs on the floor and we have the *metamorphs* that tadpoles have developed into in these cages here in the lights and we have the room set at a very specific temperature. This is called amphibian husbandry and because there are many, many species that are infected by these fungi, we have to be able to keep lots of these species in captive breeding settings; zoos or laboratories so that if they

do go extinct in the wild, then we still have an archived population that we could potentially reintroduced into the wild.
20:59 That is very tricky, so say *Chytrid* got to the island of Madagascar where there are over 500 species of amphibians found nowhere else on the planet, what do you do? Well, you can take those animals, say the Madagascan tomato frog and you can put it into a captive breeding setup such as this but then you have got to convince it that it's happy enough to breed, you have got to figure out what food it eats, you have got to make it happy and healthy and have enough individual, so you preserve it's genetic diversity so that you have got a chance of releasing it into the wild.
21:33 Many of the lessons that we are learning from keeping a typical species such as these midwife toads in this laboratory are applicable to these rapid reaction and urgency projects when *Chytrid* gets into new species, new populations and places where we don't want it to be. There is a great need to learn more about how to keep exotic species of amphibians so that we can develop these captive arks.

Q. 22:04 How much does it cost to take a new species into a laboratory and to figure out enough about it husbandry that we can keep it safely for the future?

22:22 It costs the same amount as it does to keep an Elephant for 1 year in captivity. So the cost to keeping a new species of amphibian in a laboratory is about the same as it costs to keep 1 Elephant in captivity for 1 year. So for the price of that single Elephant kept in a zoo, you could save an entire species of amphibian from extinction. I would say that, that's good return on our money.