Scientia

CELEBRATING AND SUSTAINING LIFE ON EARTH

EXCLUSIVES:

- The British Ecological Society
- The Galapagos Conservation Trust
- The Society for Conservation Biology

HIGHLIGHTS:

- Why Field Studies Matter for Teaching Biology
- Sulphur Metabolism on the Anaerobic Earth
- Investigating Metals in the World's Invertebrate Animals
- Improving Plant Disease Resistance: Can Nanoparticles Deliver?

Issue: #113

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WELCOME...

In this exciting new edition of Scientia, we celebrate our living planet, by showcasing a diverse collection of research projects dedicated to understanding life on Earth, and devising new ways to preserve and restore it.

To open the issue, we have had the pleasure of speaking to Dr Hazel Norman, Executive Director of The British Ecological Society, who tells us about how the Society communicates world-leading ecological science, promotes diversity and collaboration within the field of ecology, and much more. Next, we meet Professor Thomas Fleischner and his colleagues who are working to highlight the importance of field studies for teaching biology. His team has come up with several steps that can be taken to ensure a vibrant future for field-based education.

After this introduction, our first full section in the issue showcases research dedicated to uncovering the origins of life. Here, we feature a diverse collection of projects – from studying how minerals played a role in the emergence of life on Earth, to pinning down the habitable zones of different stars in the search for life elsewhere in the galaxy.

Our next section focuses our home planet's great biodiversity – from a species of algae that records climate change, to prairie dogs who display a rich tapestry of surprising social behaviours. Unfortunately, our planet's rich diversity is severely under threat, primarily due to human activities causing widespread habitat loss, climate change and pollution. In fact, many scientists agree that we are currently in the midst of a mass-extinction event – aptly termed 'the Anthropocene extinction'. Thus, protecting and restoring Earth's habitats is more important than ever before. Therefore, our third section in this edition showcases the tireless efforts of many scientists – each striving to preserve and revive life on Earth.

One of the most significant ways humans negatively impact the planet's biodiversity is through our agriculture. Thus, many researchers are working hard to find more sustainable ways for us to produce food, whilst also ensuring food security into the future. This is the theme of the final section in the edition, where we highlight the latest innovations in agricultural science. From finding environmentally friendly alternatives to pest control, to improving the health and wellbeing of our farm animals, the research teams featured here are making a sustainable future a reality.

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Meet The Team...

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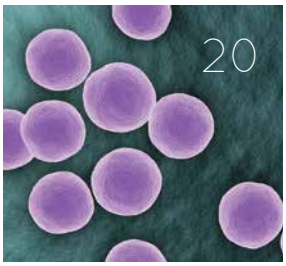
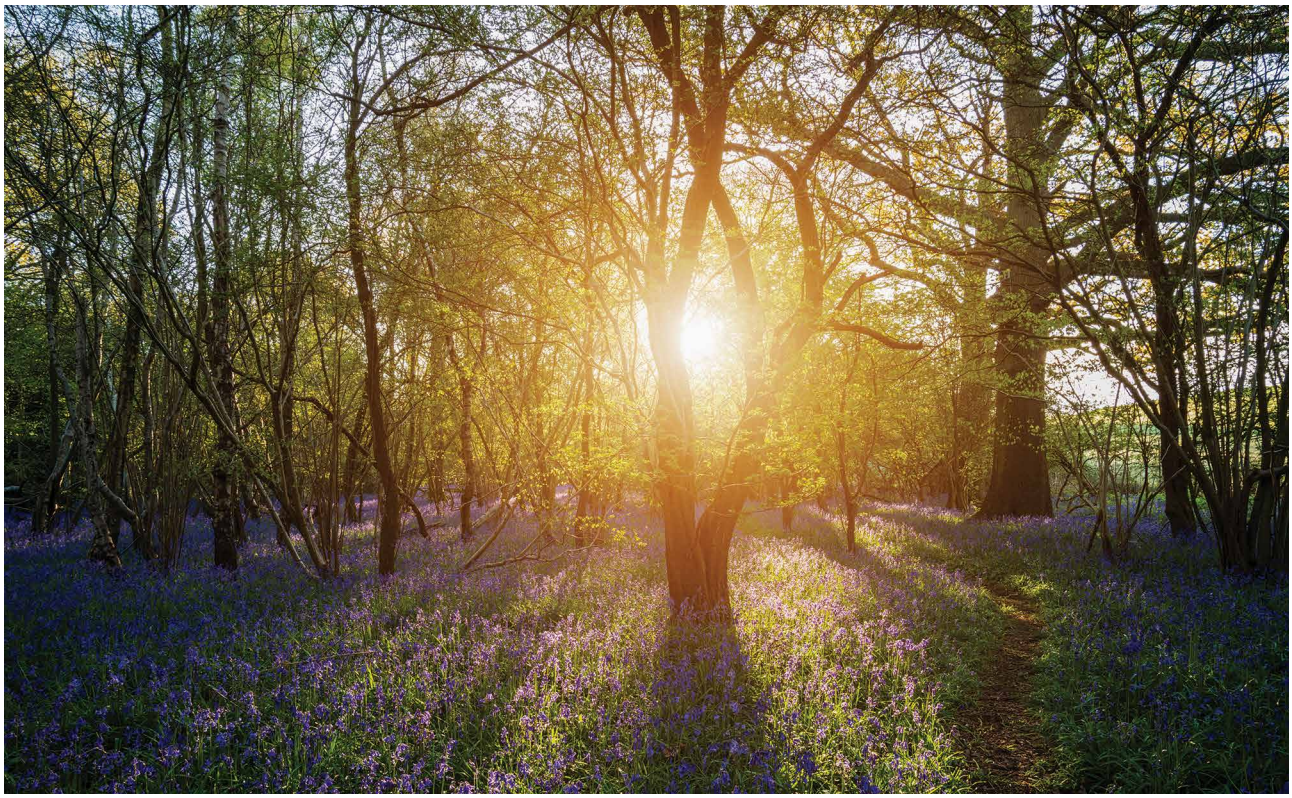


BRITISH ECOLOGICAL SOCIETY

THE BRITISH ECOLOGICAL SOCIETY

Since 1913, the British Ecological Society (BES) has been fostering the development of ecology and ecologists, with the vision of creating a world inspired, informed and influenced by ecology. With over 5000 members across the globe, the Society's mission is to generate, communicate and promote ecological knowledge and solutions. This is achieved through bringing ecologists together to share and develop insights, facilitating communication between ecologists and the public, providing evidence for policy makers and supporting ecologists throughout their career. In this exclusive interview, we have had the pleasure of speaking with Dr Hazel Norman, Executive Director of the BES, who tells us about how the Society facilitates collaboration, communicates world-leading ecological science, promotes diversity, and much more.

‘As Brexit unfolds, we mustn’t forget that ecological issues – from climate change to invasive species – do not respect borders. These global challenges cannot be solved without international collaboration.’



Since the founding of the British Ecological Society, just over a century ago, the Earth has witnessed a devastating decline in biodiversity, caused by human activity. Over this time, how has the Society evolved and changed its objectives in response?

Ecology is the scientific study of the distribution and abundance or organisms, the interaction among organisms and their environment, and the structure and function of ecosystems. Ecology provides knowledge and evidence on the interdependence between the biosphere and people. A better understanding of ecological systems enables society to predict the consequences of human activity on the environment and the importance of the environment for human welfare.

In the early days of the British Ecological Society, activities were very much focused on the academic community and publishing research. Ecology was establishing itself as a discipline and the Society supported the academic community in developing and refining the field. As ecology matured, the science provided evidence and understanding of the impact of human activity on natural systems. The Society continued its vital work in supporting the discipline and ecologists, but expanded it work outside the subject, principally to policy makers, young people and the wider public, to help them understand how important ecology is and what is happening to the natural systems that we all depend on for our health and wellbeing. All of our work is based on evidence and we are not a campaigning organisation.

Our policy, education and public engagement work are now very important elements of our strategic objectives and we commit significant resources to support them each year. As well as directly delivering projects and programmes in these areas, we provide training opportunities and support to develop the capacity of ecologists to engage in policy, education and public engagement.

Another thread that has evolved over the years is increased work across different disciplines. Global problems are usually multifaceted and require input from many areas to solve. In addition, scientists are increasingly being asked to work in multidisciplinary teams. In response to this, we have expanded the coverage of its journals, increased the subject range of its Special Interest Groups and increased the variety of topics discussed at our meetings.

Describe some of the ways that the Society works to catalyse innovation in ecological science, and how it facilitates collaboration between different groups.

The British Ecological Society is great at facilitating networking opportunities to discuss and brainstorm, and we do that in a myriad of ways. Our grassroots level organisation of Special Interest Groups means that ecologists with shared interests can communicate, collaborate and support each other in ways which directly meet the needs of those communities. Our meetings portfolio includes a huge range of different events from small groups visiting ecological sites that present particular ecological challenges to conferences like our Annual

Meeting with 1,200 delegates discussing the latest advances in ecological understanding. We actively use social media to stimulate conversations and expand our reach; we have 15k followers on Twitter and 6k global followers on Facebook.

We have a grants programme to fund research and help ecologists share their ideas through our training and travel scheme. Last year, we gave grants to 49 research projects and enabled 63 early career ecologists to present their research at meetings across the world or take part in specialist field training.

Our *Journal of Applied Ecology* publishes high-impact papers on the interface between ecological science and the management of biological resources. The journal brings together applied researchers and practitioners within ecology as authors and readers, and highlights the management implications of all its articles. However, it remains challenging to ensure that the ecological research we publish is accessible to those ecologists working outside of academia. To this end, we have conducted focus groups, a survey and interviews over the past 12 months to identify the main barriers for this audience. We are also attempting to address some of these with lay summaries and blog posts describing the journal content, offering more opportunities for practitioners to produce short papers communicating their research needs or highlighting their own work, and have an open call for Associate Editors specifically focusing on appointing non-academics to the Board.

Another way in which we facilitate collaboration is through our headquarters office in London, Charles Darwin House, which is an innovative partnership between 5 bioscience organisations interested creating space for those from different disciplines to share ideas and networks. The British Ecological Society was a founding member of Charles Darwin House which now includes the Biochemical Society, the Society for Experimental Biology, the Royal Society of Biology and the Microbiology Society along with other bioscience organisations that share the space with us.

Tell us a bit about the Society's work in communicating world-leading ecological science, both within the ecology community and to the public. What are the ways in which you achieve this?

We believe that communication is a two-way process and that our role is to facilitate this. We fund, train and facilitate our members not to communicate to, but with public groups. Our public facing activities are intended to inspire the public to engage with research and researchers; the real value for researchers comes from the opportunities to share their science and to hear the questions public groups are interested in.

The British Ecological Society publishes five world-renowned ecology journals and partner with our publisher Wiley on a sixth fully open access journal. As well as publishing over 1,000 academic articles through these journals each year, we help authors promote their work in a number of innovative ways to a wider audience: through social media, lay summaries, blog posts, videos and podcasts. Our Press Officer selects those articles that they believe will have the widest general appeal and promotes these via press releases to media outlets all over the world, including the BBC, CNN, ABC News, *The Times, New Scientist* and countless others. Authors of press released papers are also encouraged to do interviews with journalists and have featured on national radio and TV channels to promote their research.

Through what means does the Society help school children foster an interest in ecology, and how do you guide undergraduate students towards a rewarding career in ecological science.

Biology is a broad subject area, within which ecology is one aspect. At primary and lower secondary level, we collaborate with other bioscience organisations to recommend the curricula content children need to learn at each of their education stages. A common thread for all bioscience societies is to highlight the importance of practical science and within this, the British Ecological Society is a strong voice seeking to ensure that children are able to access field based practical experience. As students move through the education stages we begin to focus more strongly on ecological interventions, developing resources and working with awarding bodies to ensure ecology is represented within qualifications.

We work with A-level students, specifically from black and other minority ethnicities, lower socio-economic and those who are first in their family to consider higher education, to encourage them to take up further study in ecology. We do this through career advice and by including a group of these students on the annual BES Summer School.

The Summer School is in its 3rd year

and provides an opportunity for 50 Undergraduates to come together and spend a week focussing entirely on ecological content, future careers and building a network of likeminded individuals. The School's real strength lies in the mix of Pre-University, Undergraduate and PhD student mentors. Not everyone who applies can attend the Summer School, but those who do attend form the organising committees of our careers conferences and local representatives and in so doing are able to share their experiences.

Once on the path to a career in ecology, what support is available for early career ecologists?

Early Career is a difficult term to pin down. The Society really uses the term to define anyone who's still seeking a permanent post. Open to all early career ecologists, we offer a wide range of training and support. We encourage early carer representatives across all our committees and working groups, they interact with each other through an early career working group which acts as the expert panel advising on the training and support we offer. We have a place on our Board of Trustees specifically reserved for an early career ecologist to ensure their voice gets heard at the highest levels within the organisation.

Much of the technical and discipline specific training is accessed through our Special Interest Groups, each has a student and early career representative ensuring that their needs help shape activities. Non-research specific training is offered centrally from the Society in science communication through hands on projects with schools and the public. We also run various mentoring schemes; including specific schemes for women in ecology and new associate editors. Grant writing courses are delivered each year for those seeking post-doctoral fellowships and research grants. We produce a range of guides and run workshops on various key skills for researchers, including how to get published, how to peer review and data management.

We also offer training and travel grants, and other early career grants that support our members to attend and engage in opportunities outside the British Ecological Society. The Journals each award an annual prize for the best paper published by an early career researcher. This prize entitles the author to present their research at the Annual Meeting as well as covering their registration costs and a cash award.

'Our public facing activities are intended to inspire the public to engage with research and researchers; the real value for researchers comes from the opportunities to share their science and to hear the questions public groups are interested in.'

How does the British Ecological Society work to promote a culture of diversity, equality and inclusion?

In our latest strategic plan, we identified a need to ensure that we were an open and inclusive organisation and discipline. So, in 2016, we established an Equality and Diversity Working Group to help us ensure that a career in ecology is accessible and welcoming to anyone, irrespective of their background. During the year, we developed our equality and diversity policy for the organisation, which is published on our website, and developed a set of equality and diversity guidelines for all our Committees, working groups and SIGs. These guidelines cover issues such ensuring opportunities are open to all, and that speakers at BES events should represent the full diversity of the ecological community. We launched a new prize – the BES Equality and Diversity Champion, to recognise an individual or group who have campaigned to highlight the importance of equality and diversity and worked to make a difference or served as an inspiration to others. We also use our Annual Meeting as a platform for an inclusive community, for example by providing space and publicising LGBT+ group and Christian group meetings, providing a prayer room and providing a family room. We have started systematically collecting equality and

diversity data on our members and those who use our services so we can make sure we really are accessible to all equally. To demonstrate our long-term commitment to improving equality and diversity in the ecological community we have pledged to report annually on our progress and you can find our first report at: http://www.britishecologicalsociety.org/about/diversity-and-the-bes/

Tell us about the Society's work in supporting ecologists in Africa. Describe one or two projects that the Society is currently involved with.

We have been targeting support for ecologist in Africa for several decades, partly in partnership with others.

One of our research funding streams, 'Ecologists in Africa', provides support of up to £8,000 per grant for ecologists in Africa to carry out innovative ecological research with an optional additional £2,000 to fund travel to help them develop connections with other ecologists outside their usual peer group. We recognise that ecologists in Africa face unique challenges in carrying out research and our grant is designed to support African ecologists develop their skills, experience and knowledge base as well as making connections with ecologists in the developed world. We made 7 awards in 2016.

Another way in which we support ecologists in Africa is in partnership with the Tropical Biology Association (TBA). We provide a grant of £10,000 per annum to help the TBA deliver its innovative field course programme. The TBA runs month-long courses in tropical ecology and conservation for students at advanced undergraduate or early postgraduate level who have a keen interest, but little experience, in tropical biology. The courses provide practical training and experience in the tropics with an emphasis on building skills and understanding. They provide a valuable foundation on which to build a career in conservation, research and sustainable natural resource management. On every course, participants represent at least 15 countries, from the host region and beyond, ensuring a rich cultural experience, and laying foundations for a lasting conservation community that spans the globe. They are truly transformative experiences for the participants and the BES funding helps to offset the costs.

Finally, does British Ecological Society influence policy decisions, and how? How do you believe Brexit will affect the Society's work, and what challenges could it present for science and the environment over the next 10 years?

Our approach to influencing policy has members at its core. We run a variety of initiatives to enable members to develop their policy engagement skills and experience, ranging from training workshops to placements in Parliament. Then we draw on the collective expertise of our membership to bring together the best ecological evidence on wide range of policy and communicate that to decision-makers. Much of this work is done in partnership with other scientific and environmental organisations.

Brexit is already having a substantial impact on our work and that of our members. However, it is really important for us that Brexit doesn't mean restricting our horizons. We are an inclusive, international society, and we remain committed to fostering cross-border collaborations and representing all our members, wherever they may live.

Brexit will bring about major changes to our environmental policy framework, and has put UK science and scientists in a position of real uncertainty. There are major risks ahead, but also opportunities, and the ecological community must engage proactively to be heard. Since the referendum, we have been incredibly busy: meeting with Ministers, giving evidence to Parliament, and holding a number of high profile events to highlight our concerns.

Looking ahead, maintaining the UK's position as a world leader in science is essential, which means an immigration system that maintains our ability to attract the best staff and students from a global talent pool, and securing the best possible access to European collaborative networks and funding. It is vital that environmental standards are maintained, or better, improved, and that any legislative changes as a consequence of Brexit are informed by the best ecological evidence.

As Brexit unfolds, we mustn't forget that ecological issues – from climate change to invasive species – do not respect borders. These global challenges cannot be solved without international collaboration.

BRITISH ECOLOGICAL SOCIETY

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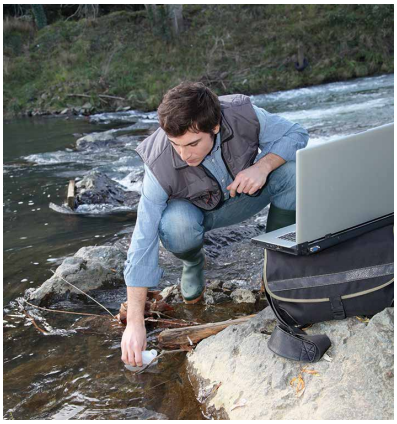
WHY FIELD STUDIES MATTER FOR TEACHING BIOLOGY



Professor Thomas Fleischner and his colleagues at the Natural History Institute at Prescott College, and across the United States, are working to highlight the importance of field studies for teaching biology. Here, we discuss why the decline of field studies should not be taken lightly, and several steps that can be taken to assure a vibrant future for field-based education.

Traditionally, field studies have been an essential component of the pedagogical toolbox, but in recent years their use has seen steady decline. Field studies are crucially important, especially in the biological and earth sciences.

Professor Thomas Fleischner is a Professor and Director of the Natural History Institute at Prescott College, Arizona, where he teaches natural history, ecology and conservation biology. In addition to his work in environmental studies, he frequently works at the fringes between disciplines, looking at the interactions between science and public policy. He believes that field-based education is a crucial part of a biologist's training, and that natural historians have a responsibility to assert its importance.

Why is field education important?

To use an analogy, learning to play the piano involves listening to the music of others and an understanding of music theory. However, these aspects alone are of little use to an aspiring pianist without them physically putting their fingers to the keys and practising. Similarly, in the life sciences, there is a difference in the way students contextualise information when they observe life in its natural environment, rather than in the laboratory. In many biological subjects, the lab is appropriate and entirely necessary, but for others, such as the study of animal behaviour, there is no substitute for observations made in the animal's natural habitat. These experiences can instil a far greater connection between students and the biological world, compared with those in the lab or the classroom. The complexities of evolution and ecology are also better appreciated when observed directly in the context of the natural world, rather than – for example – in a schematic diagram of a food-chain.

In addition to learning directly from the natural environment, students learn more fully and more deeply from their instructors and each other, as well as local residents and working professionals. Students are often more receptive to novel experiences when taken out of their usual environments, with analyses showing that content and skills are often better retained following field-based courses compared to lab-based ones. It has also been recently reported that 'interesting course content' was the single most important factor behind students choosing

Professor Fleischner and colleagues argue that some of the most pressing socio-economic issues, and their solutions, lie at the intersection between culture and nature – where field biology takes place.

field courses. So not only do students appear to learn more effectively on field courses, but they also enjoy them. Therefore, it is not an unreasonable logical leap to suggest that these factors are almost certainly linked.

In the face of climate change and the loss of biodiversity, society often looks to scientists to mobilise appropriate responses. If upcoming generations of biological scientists are increasingly divorced from a connection with nature, their responses to these issues might be lacking in enthusiasm and be less effective. Increasing the use of field studies in college courses could be an ideal way to better instil this connection in our future scientific leaders.

Problems and Solutions

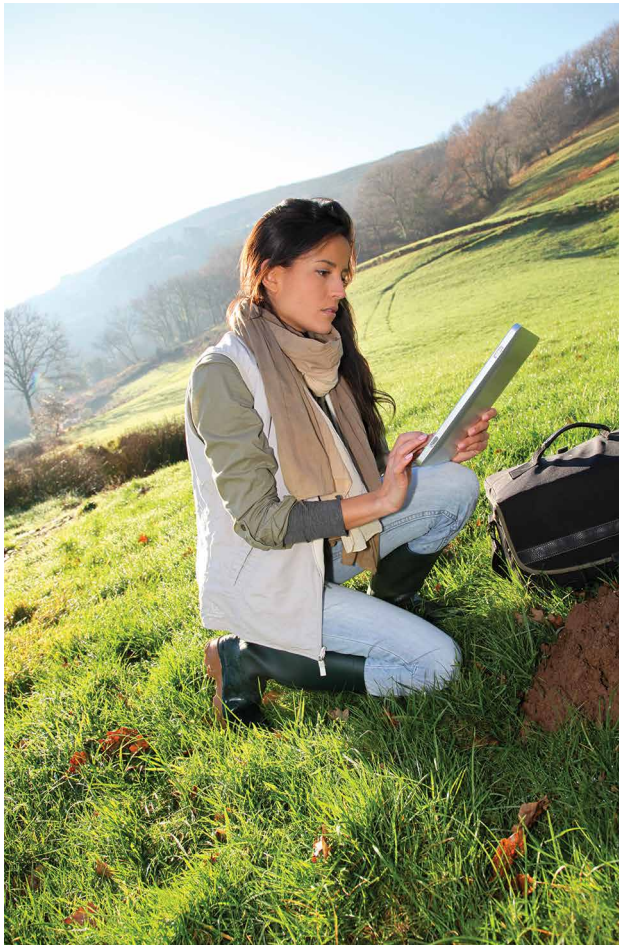
Concerns regarding risk assessment in various aspects of life can often seem tiresome. Though annoying, risk assessment is usually implemented for people's benefit. However, the extra paperwork involved in organising a field-trip to the jungle for 30 students is not a trivial matter for an already overworked academic, and is undoubtedly a factor when deciding whether or not to conduct a field course. This aversion to health and safety paperwork is one factor, in addition to the logistical, financial, and legal challenges of organising a field-based course, that contributes to their decline.

Although the demand for field-based studies often exceeds supply, many life-sciences students mistakenly believe that field studies are less useful in enhancing employability, compared to laboratory-based classes. Student attitudes may also be partly to blame for declining field studies when we consider the way the world is becoming increasingly urbanised, and with childhood exposure to nature diminishing. Modern students are perhaps less likely to feel a connection to the natural world outside of cities, and may feel that environmental issues are irrelevant when compared with those of social justice or the economy, in spite of the inseparability of social and environmental challenges. The decline in the use of field studies at some institutions may be reflective of these societal shifts, and undergraduate participation in field studies may often be unrepresentative of real cultural diversity.

Professor Fleischner and colleagues argue that some of the most pressing socio-economic issues, and their solutions, lie at the intersection between culture and nature – where field-biology takes place.

While students from rural or even suburban upbringings may feel little concern about the challenges associated with a field-based study, students from an urban environment, who may never have spent a night in a tent, may find the prospect of field work to be a source of anxiety. Female students may also have concerns if the class consists of a male majority, while students with disabilities could be discouraged from partaking if their disabilities are difficult to accommodate.

Some of these issues are at least partly culture-specific. Professor Fleischner and colleagues note that scientists and institutions in North America have been slow to acknowledge declining field-based experiences, compared to those in the United Kingdom, for example. However, as of March 2016, the US National Science Foundation funded Professor Fleischner and the Natural History Institute to convene a working group



with the specific purpose of addressing this issue and offering potential solutions. The project's title was *'The Decline in Field Studies: Proactive Strategies for Essential Training for the Next Generation of Biological Researchers.'*

With regard to changing student attitudes, the teaching of field courses needs to become more relevant and accessible in our rapidly changing world. All students should feel welcome to take part in field-based courses designed to acknowledge, respect, and accommodate their various backgrounds and needs. Professor Fleischner and colleagues feel that this will not only benefit the students who broaden their horizons by taking part, but that the field courses themselves will be enhanced by these diverse perspectives.

Teaching staff may be enthusiastic about field-based education, and appreciate their importance to the personal development of students, but at the same time feel unqualified or lacking in confidence to organise and lead such activities. Aside from the logistical technicalities, the pedagogical techniques required in a field setting are different from those needed in the classroom. Teachers, like students, require guidance and support, and those who have experience in this area should take the initiative in mentoring their less experienced colleagues. Institutions hoping to increase their use of field studies would need to ensure that academic staff are granted the time required for this mentoring and development.

Rather than decrying the logistical and risk-assessment hurdles of field study preparation, Professor Fleischner and colleagues hope that researchers will become more engaged in the conversation at the institutional level, and approach these regulatory aspects from the ground up. Easy access to training should be provided, to teach course organisers the relevant skills required, such as risk management and dealing with interpersonal issues like sexual harassment and cultural tolerance.

Risk management can also be a problem at an institutional level, with the college often being unwilling to accept the liabilities associated with field-trips. Institutions may not be aware that a wealth of expertise and 'best practises' exist in the world of adventure education. Professor Fleischner's group has prepared a manual (*Saying Yes to Environmental Field Studies: A Guide to Proactive, Successful Administration*), which includes relevant field protocols based on adventure education models, to support this approach. This is publicly available at http://naturalhistoryinstitute.org/field-studies/

Professor Fleischner and colleagues propose that not only should all biology students be required to complete a field-based course during their studies, but also that teaching staff should attempt to teach at least one field-based course during their career. One incentive for teaching staff could be for institutions and professional societies to create distinguished teaching awards specifically for field-based instruction, not necessarily limited to the life-sciences. Institutions could also modify budget allocations to include a mixture of classroom, laboratory and field-based experiences on appropriate course modules, and revise curricula to ensure students engage in all three settings.

Redefining 'The Field'

When considering whether to run a field course, many instructors will not stray too far from a generally accepted concept of what constitutes 'the field'. Professor Fleischner and his colleagues believe that it would be of great benefit to expand this definition to include more diversity, such as urban neighbourhoods, feral fields, zoos and botanical gardens. Professor Fleischner and colleagues assert that restriction of 'the field' to wildland settings may be both unrealistic and irresponsible, and that expansion of its definition could help with the logistical, economic and institutional hurdles described above. He suggests that rather than venturing all the way to the wilderness, observations can be made in an urban setting. This could include evaluating the diversity of pollinating insects in urban farm plots, studying foraging peregrine falcons, recording the dawn chorus of birds and camera trapping rodents and other urbanised mammals. While these experiences might still be limited in comparison to studies performed in a more traditional field setting, they can at least pique the interests of students and teach them the basic skills required for field study, such as observation, recording data, interpretation and exploration of nature. Teaching students about the nature around them in urban and suburban environments, and how their investigations can answer fascinating biological questions can help them to re-envision these places as being inclusive in nature. This realisation that nature is not only something that occurs outside of a student's immediate environment can help build their connection with the natural world and engage with the life sciences in a way they might not otherwise if they learned only from the classroom and laboratory. In order to prevent, and even reverse their decline, field studies need to become more available, inclusive and relevant to the modern world.

Meet the research team leader

Professor Thomas Fleischner

Executive Director – Natural History Institute; Faculty Emeritus

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Professor Thomas Fleischner completed his first degree in Field Biology in 1977 at The Evergreen State College and achieved his PhD in Environmental Studies in 1998 at The Union Institute. He co-founded the North Cascades Institute in Washington State and is currently Director of the Natural History Institute and a Professor at Prescott College, Arizona. Here, he has taught in the interdisciplinary environmental studies program for the last 29 years. In addition to courses in natural history, ecology, and conservation biology, Professor Fleischner's teaching covers a broad range of disciplines including creative writing, environmental politics and ecopsychology. He has written two books – *Singing Stone*: *A Natural History of the Escalante Canyons and Desert Wetlands*, and edited two others, *The Way of Natural History*, and *Nature, Love, Medicine: Essays On Healing In Wildness*, which is currently in press.

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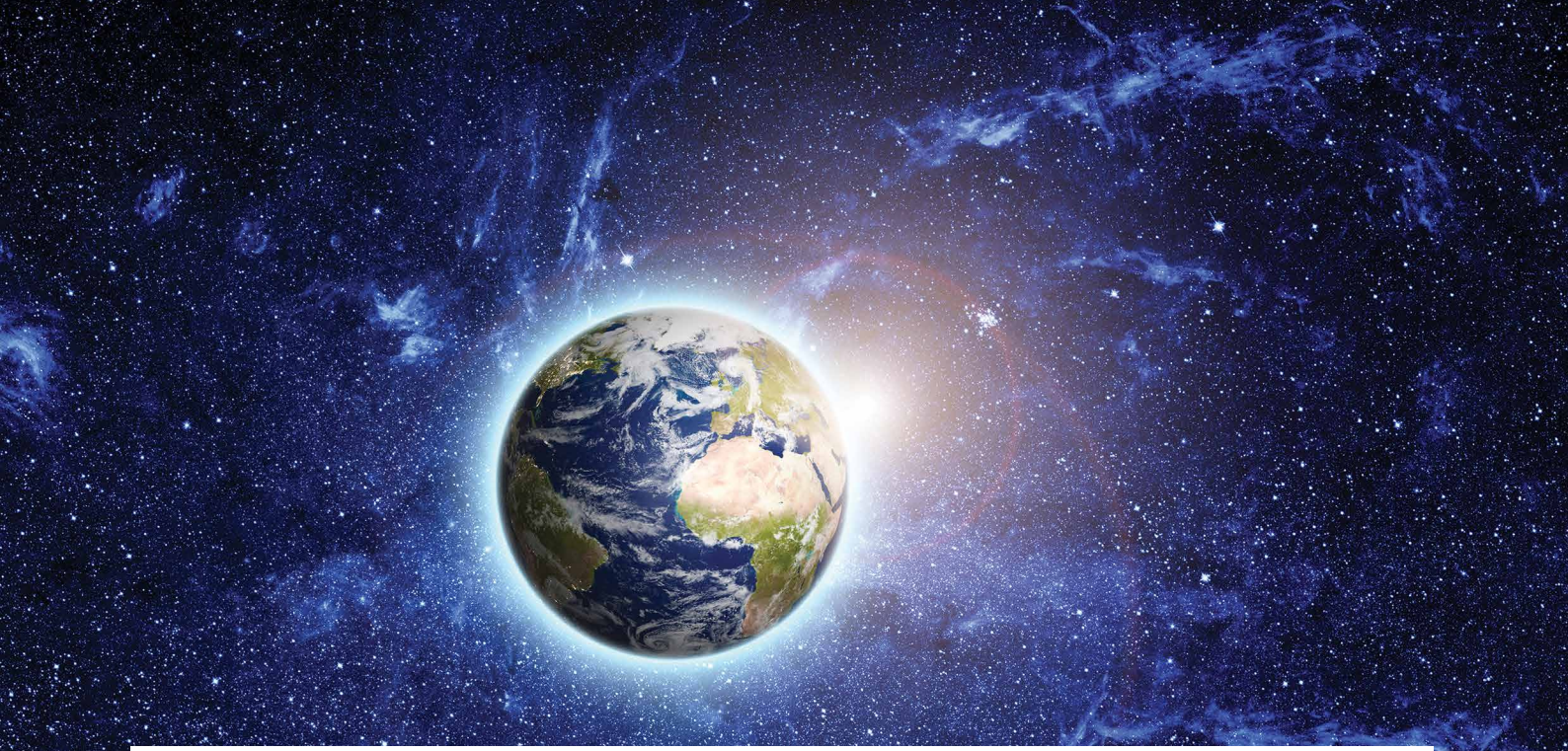
http://naturalhistoryinstitute.org/field-studies/



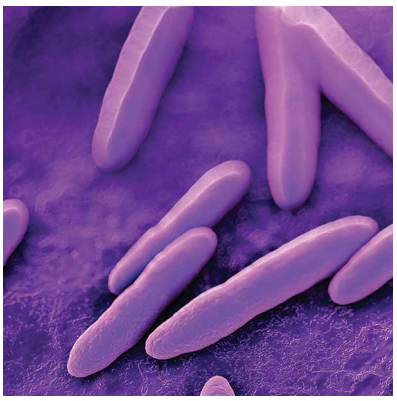
HOW DID LIFE BEGIN?

Our planet is home to a diverse array of lifeforms, with each organism striving to survive and multiply. In one famous study published in *PLOS Biology* in 2011, scientists estimated that the number of eukaryotic (non-bacterial) species currently living on Earth is 8.7 million, give or take 1.3 million. However, adding the prokaryotic organisms to the mix – bacteria and other single-celled organisms called Archea – the number of distinct species was recently estimated at a whopping 1 trillion. This argument, put forward by a team of scientists at Indiana University, was published in the *Proceedings of the National Academy of Sciences* in 2016. If true, their estimations would mean that 99.999% of all the species on Earth are currently undocumented.

So how did life on Earth first begin, before diversifying into this enormous multitude of organisms – from bacteria to fungi, and from plants to animals? How did non-living molecules first come together to create an entity that could self-replicate? Are all organisms on Earth descendent from one single common ancestor, or does life have multiple origins? These questions are still being debated by geophysicists, chemists, biochemists and palaeontologists alike.

One of the first proofs showing that biological molecules can arise under the physical conditions present early in Earth's history was an experiment conducted by the American chemists Stanley Miller and Harold Urey in 1952. In their famous Miller–Urey experiment, the pair demonstrated that amino acids – the building blocks of proteins – could be created by subjecting a mixture of water, methane, hydrogen and ammonia to electrical discharges. Soon after, the Catalonian biochemist Joan Oró showed that by adding cyanide to Miller and Urey's 'primordial soup', the compound adenine could be synthesised. Adenine is one of the four nucleobases found in DNA, which, along with guanine, cytosine and thymine, forms the blueprints for all organisms on Earth. Indeed, in later experiments, researchers showed how these other nucleobases could emerge, under the conditions believed to be prevalent on the early Earth.

Rather than originating on Earth, many scientists believe that these pre-life – or 'prebiotic' – compounds might have been synthesised in space, being delivered to the Earth on meteorites during the Late Heavy Bombardment – a period in which a disproportionately large number of asteroids are theorised to have collided with Earth and other planets. Indeed, scientists have found nucleobases on many meteorite



samples, although it is difficult to confirm that their presence is not due to terrestrial contamination. In one particularly convincing study published in *PNAS* in 2015, Italian and Russian scientists showed how the compound formamide (which is abundant throughout space) could be catalysed by meteorite material and the solar wind to afford not just nucleobases, but also amino acids and sugars. These and other similar findings hint that life on other planets may not be all that different from life on Earth.

Although the origin of these prebiotic compounds is still the subject of debate, how the first complete cell emerged from these basic building blocks is perhaps even more uncertain. The protocell – a hypothetical

simple cell-like entity – is believed to have preceded the earliest cells, and is thought to have emerged between 3.8 and 4.2 billion years ago, likely in the vicinity of hydrothermal vents – hot springs on the ocean floor. The protocell may be described as having the minimal characteristics shared by all forms of life, such as genetic material (some primitive form of DNA), an enclosing membrane and a metabolic system.

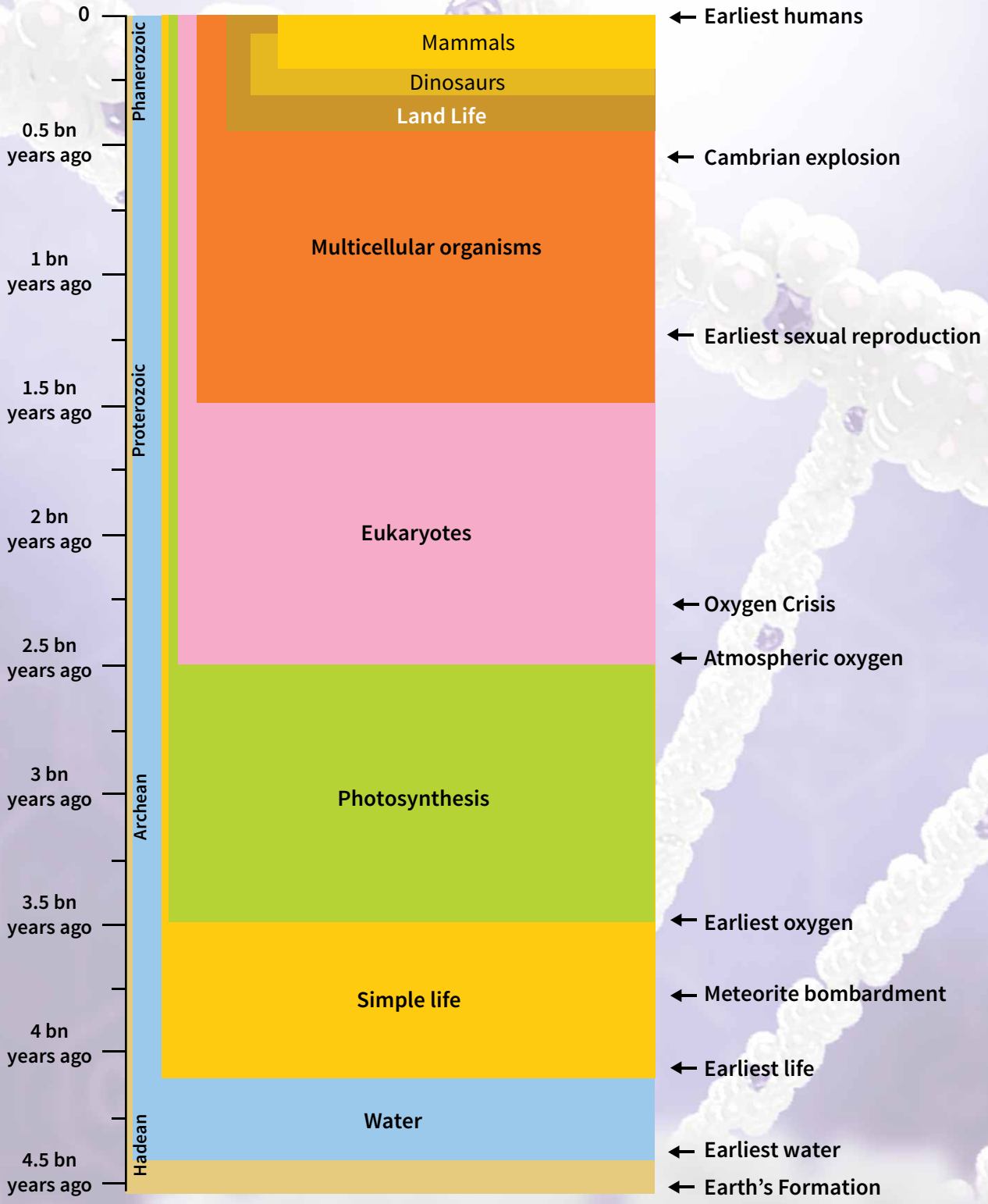
This protocell – perhaps the common ancestor of all life on Earth – is what Dr Nita Sahai, of the University of Akron, focuses her research on. In this section of the edition, we introduce her work, investigating the geochemistry behind protocell formation and evolution, with a view to understanding the origins of life on Earth. As mentioned above, one of the key components of the protocell is an enclosing membrane – a sheath composed of lipids that encloses and protects the other cell components. These lipids can spontaneously form into layers and spherical vesicles under the right conditions. Dr Sahai investigates how mineral surfaces might have played either a stabilising or disrupting role in the formation of a protocell membrane from such lipid molecules. This fascinating research is helping us to understand not only how life might have started on our own planet, but also how it may arise on others.

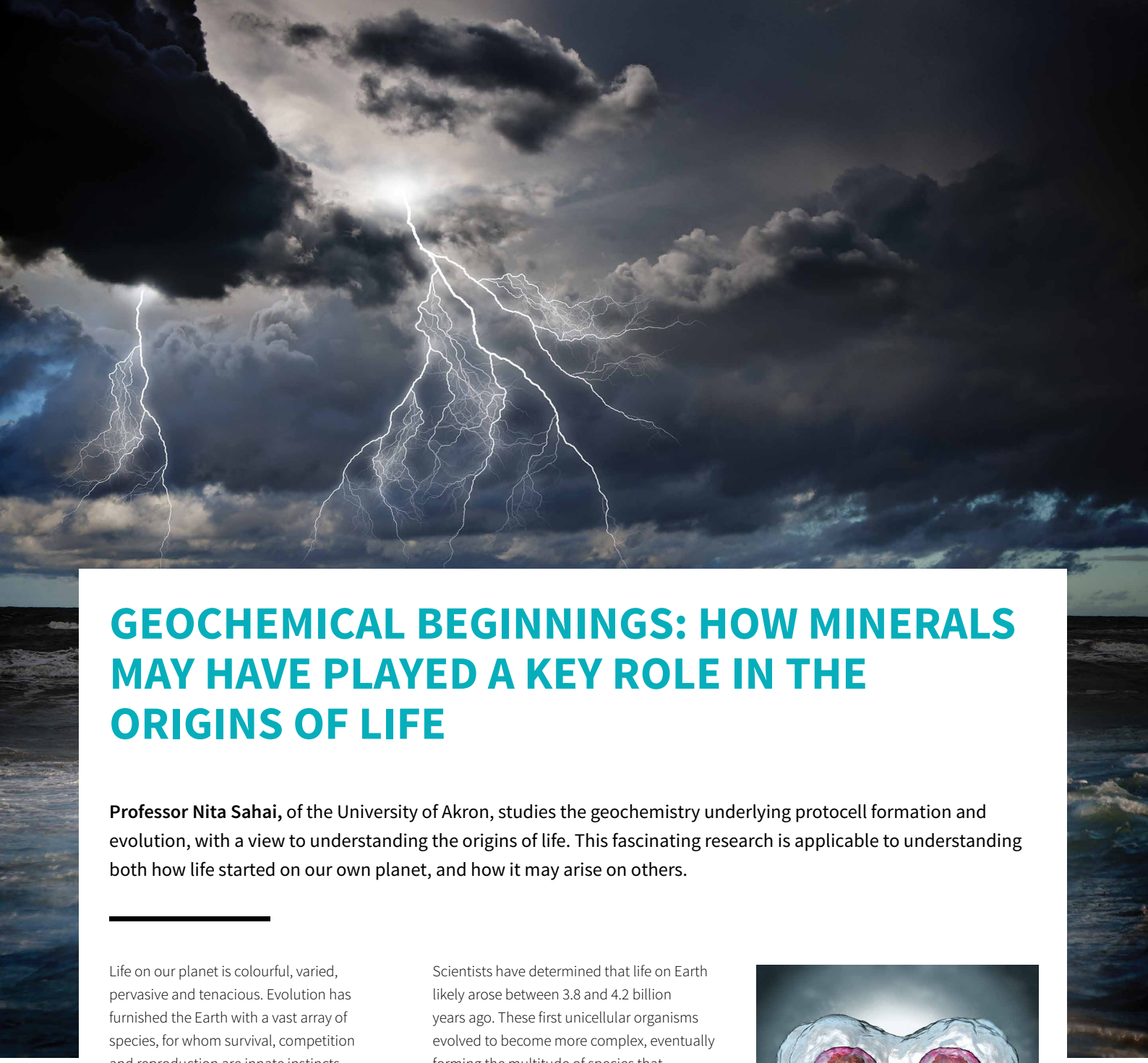
Next, we feature the research of Dr John Perona and his team at Portland State University, who study the biochemistry of methanogens – microorganisms that evolved on the early Earth and are still prevalent today. Since only very small quantities of molecular oxygen were present on Earth soon after its formation, the earliest living cells respired anaerobically, relying on chemical processes that are found today only in extreme conditions where oxygen is still absent – such as the intestines of animals and humans. Methanogens are of particular relevance today as their sulphur-dependent metabolism produces the greenhouse gas methane – a key driver of global warming. Thus, improving our understanding of methanogens could have practical implications for addressing climate change. Also, similar to Dr Sahai's work, enhancing our understanding of ancient life will offer insight into how life might develop on other worlds.

Also studying the metabolic processes of primitive microorganisms are Dr Costantino Vetriani of Rutgers University and Dr Frank Robb of the University of Maryland. Here, we showcase their work on thermophiles – microorganisms that thrive at high temperatures, such as those that live near hydrothermal vents. The team is shedding light on the biochemistry of these important marine environments, believed to have been home to the Earth's very first lifeforms. In addition, they are unravelling the evolutionary relationships between deep-sea vent microorganisms and disease-causing bacteria, thus providing insight into how virulence arises in human pathogens.

Of course, the protocell and its decedents – the primitive microorganisms studied by Drs Perona, Robb and Vetriani – could never have emerged if it wasn't for the presence of water on Earth. This is one of the many research focuses of Dr Jim Kasting, who defines and assesses the 'habitable zones' of stars – the distance at which liquid water might exist on the surfaces of orbiting planets. Along with his colleagues at Penn State University, Dr Kasting has been studying stars and the boundaries of their habitable zones for decades. His work is now informing the design and construction of the next generation of space telescopes, which will hopefully lead to the discovery of life on other planets.

TIMELINE OF LIFE ON EARTH





GEOCHEMICAL BEGINNINGS: HOW MINERALS MAY HAVE PLAYED A KEY ROLE IN THE ORIGINS OF LIFE

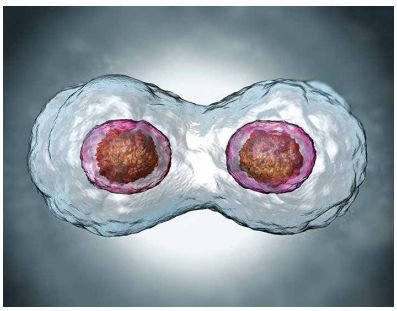
Professor Nita Sahai, of the University of Akron, studies the geochemistry underlying protocell formation and evolution, with a view to understanding the origins of life. This fascinating research is applicable to understanding both how life started on our own planet, and how it may arise on others.

Life on our planet is colourful, varied, pervasive and tenacious. Evolution has furnished the Earth with a vast array of species, for whom survival, competition and reproduction are innate instincts. These properties of life, can, under optimal conditions, drive self-replication and ever increasing biodiversity, in perpetuity. Life on Earth is programmed to survive and thrive. From microbes to mammals, from deep sea trenches to volcanic vents and frozen ice caps, life in all its forms has adapted to the myriad habitats our blue sphere has to offer, elegantly, doggedly, successfully. But where did it all begin?

The origins of life on Earth remain a fundamental and profound mystery. As a species, our consciousness and self-awareness dictate that we are inherently curious about our own origins and those of the other species that share our planet. Such thinking forms the basis of much of our art, philosophy, religion and science.

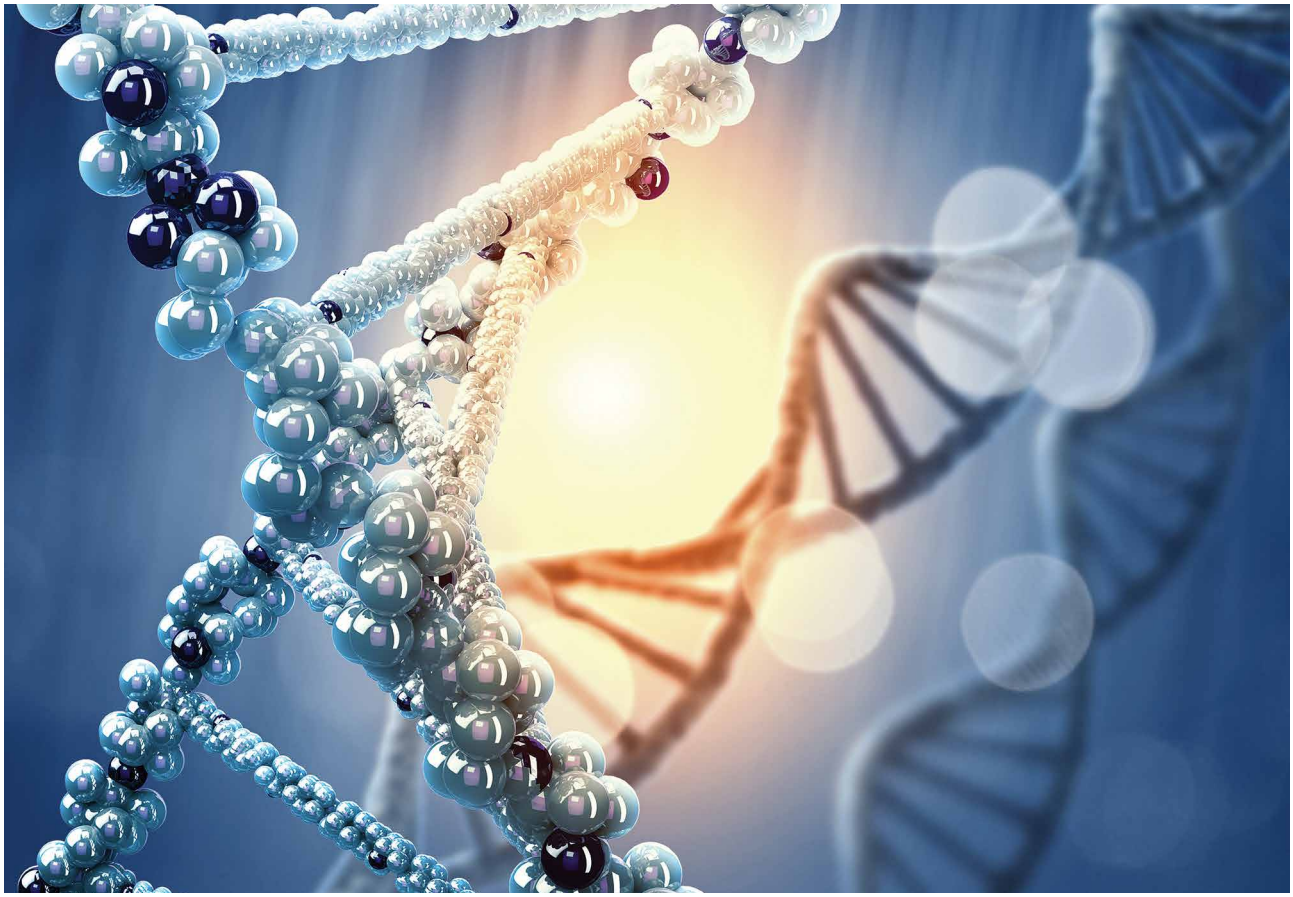
Scientists have determined that life on Earth likely arose between 3.8 and 4.2 billion years ago. These first unicellular organisms evolved to become more complex, eventually forming the multitude of species that inhabit almost every conceivable habitat on the planet. Professor Sahai spoke with Scientia about her motivations for scientific investigation into our origins: 'To me, the scientific approach is the most robust and intellectually satisfying to answering the questions about the origins of life. Finding the answers to these tremendously difficult questions, more than any other I can think of, requires the integration of knowledge from a wide array of fields such as Geochemistry, Earth History, Biochemistry, Organic Chemistry, Meteoritics, Planetary Science, Exo-solar Planets, and Astronomy.'

Put simply, scientists like Professor Sahai and her team theorise about how life could arise from non-living primitive materials, under specific conditions. In particular,



it is important that these materials, both inorganic and organic, and environmental conditions be as reflective as possible of what is thought to have existed on Earth during the period that life first arose. Determining what these building blocks were, and what conditions were required to enable them to combine and interact in ways that resulted in the inception and survival of primordial life, is a fascinating and interdisciplinary branch of scientific endeavour.

‘The over-arching goal of my research program in the Origins of Life is the bottom-up assembly of a “protocell” under realistic and plausible ranges of early Earth environments’



The Protocell

So what would this early life have looked like? A protocell, which is a hypothesized primitive cell-like entity that is believed to have preceded the earliest cells, may be described as having the minimal characteristics shared by all forms of life. These characteristics include a lipid membrane, to enclose and protect all the other components. They also include a metabolic system, which constitutes energy-transfer reactions. Such reactions must be catalysed, either by enzyme-like molecules or by other chemicals. The protocell must also have included some genetic material, to permit for hereditary transfer and reproduction, which is likely to have been a much more primitive form of DNA (deoxyribonucleic acid) and is widely assumed to be RNA (ribonucleic acid). Although we can describe the protocell as ‘primitive’, in comparison with the complex life forms that currently inhabit the planet, the conditions, materials and reactions that were necessary for it to arise from non-living material likely demonstrated a complex interplay. The chemistry required to give rise to these characteristics creates what Professor Sahai refers to as a ‘chicken-and-egg problem’. Before the existence of biological processes (such as the catalytic function of enzymes) what could have caused these reactions to occur? The protocell is thought to have evolved after the time that liquid water became stable on Earth, about 4.2 billion years ago. The first signs of life on Earth that have been detected in rock have been dated at approximately 3.8 billion years old. Therefore, the protocell is thought to have emerged at some point before this. Professor Sahai tells us about how the protocell concept is at the basis of her research, and is applicable to life on this planet and others: ‘The over-arching goal of my research program in the Origins of Life is the bottom-up assembly of a “protocell” under realistic and plausible ranges of early Earth environments, and to identify potential relationships between protocell stability and environmental conditions to predict the habitability of other solid worlds.’

Bottom-up Protocell Self-Assembly

One of the key components of the protocell is an enclosing membrane, which can spontaneously form from lipids into what is known as a bilayer membrane that encloses and protects the other components of the cell. These lipids will naturally form into layers and spherical vesicles under the right conditions. Professor Sahai investigates how mineral surfaces could have played either a stabilising or disrupting role in the formation of a protocell membrane from such lipid molecules, or affected the rate at which they form.

The influence of minerals on bilayer self-assembly has been previously investigated by a variety of researchers from different fields. However, universal relationships between lipid assembly and mineral chemistry have not yet been established. The surface properties of minerals are numerous and characterising their interactions with lipids is a complex task. Other confounding processes include difficulties accounting for

‘The multi- and inter-disciplinary nature of this research area is exciting because, for one, I am always learning something new and am living at the edge of my own area of expertise’

the influence of gravity on mineral particles, which can cause them to settle out in solution, thereby limiting their contact with lipids.

Professor Sahai and her team examine the influence of a wide range of synthetic inorganic solids, and natural minerals and rocks, on vesicle-formation rates, thermodynamic stability, and membrane permeability. The team study the crystal structure, chemical composition, particle shape and size, surface charge and solubility of minerals, with a view to identifying what are known as structure-activity relationships (SARs) between lipid membrane formation, environmental conditions and mineral properties. This means that they employ minerals of varying structure or chemistry and/or vary the environmental conditions slightly to see what effect these changes have on membrane formation. Excitingly, if such a relationship can be comprehensively established, the team may be able to predict whether cell membranes can be stable on other solid worlds. Professor Sahai tells us about how her research is applicable to other planets and how SARs are a useful tool to parse the numerous combinations of factors that are involved in these complex dynamics. ‘In the laboratory, we can only examine a limited number of minerals, solution compositions, temperatures, etc. Contrast that to the thousands of minerals known and the wide range of geochemical solution compositions and temperatures from sub-zero in the polar regions to those found at deep-sea hydrothermal vents (at around 300ºC). It would be virtually impossible to study all of them to examine whether specific organic molecules would be stable or not in all those environments,’ she explains. ‘So, identifying SARs would be ground-breaking because they may help predict the plausible self-assembly of prebiotic organic molecules and protocell emergence on other solid worlds such as Mars, the moons of Earth, Jupiter, Saturn and exo-solar planets, as their mineralogy becomes known. This is especially important for solid worlds that are too remote to send landers or rovers on to sample their surfaces or atmospheres directly.’

Early Earth Environments – a Cocktail for Primordial Life

Determining what substances were present in the early Earth environment, and what forms and levels they were present in, is also crucial to Professor Sahai’s work. Using this information to determine what effect the environment had on protocell formation is at the crux of the work. One aspect of this is looking at available phosphate in the early Earth environment. Phosphate is a component of numerous biological building blocks, such as nucleotides and phospholipids, and it is thought that much higher levels of phosphate and magnesium were required to enable the non-enzymatic (prebiotic) formation of certain biological components, such as nucleotides, compared with biological enzymatic reactions. However, levels of available phosphate on the early Earth are believed to have been hampered by the limited solubility of a calcium phosphate mineral (hydroxyapatite), a conundrum known as the ‘phosphate problem’.

Building up a realistic picture of the conditions present in the early Earth environment is important to accurately model the interactions of molecules present in such an environment. In collaboration with Professor Martin Schoonen at Stony-Brook University, New York, the team have set out to determine if biologically-relevant levels of magnesium, calcium and phosphate can exist under plausible early Earth temperatures and atmospheric levels of carbon dioxide. The team employ geochemical modelling techniques to analyse atmosphere-water-rock interactions to answer these questions.

The Origins of Life: An Interdisciplinary Discipline

The potential to undertake collaborations, such as the one described above, and the interdisciplinary nature of this research is part of what attracted Professor Sahai to this field of research: ‘The multi- and inter-disciplinary nature of this research area is exciting because, for one, I am always learning something new and am living at the edge of my own area of expertise. What I mean is that multidisciplinary work stimulates my creativity to ask questions at the boundaries between multiple fields and my own core expertise in geochemistry,’ she explains. ‘My research and that of other scientists in the origins of life research area can be mutually informed by the knowledge and open questions in the others’ fields to create potential breakthroughs that would not otherwise be possible.’

Professor Sahai is also keen to acknowledge the contributions of the students and post-doctoral scientists in her group, and the support that her team has received for their work to date: ‘This research has been funded by two NSF grants (CAREER and a current one), NASA Astrobiology Institute and the Simons Collaboration on the Origins of Life (SCoL). The Simons Foundation Collaboration of Life (SCoL) involves about 20 Principal Investigators, and we span a wide range of expertise from Astronomy and Meteoritics to Physics, Biochemistry and Geochemistry. The grant is funded for five years at a level of more than $80 million (http://www.simonsfoundation.org/life-sciences/simons-collaboration-on-the-origins-of-life/). The SCoL is directed by Dr Jack Szostak, Nobel Laureate 2009 and Howard Hughes Medical Institute (HHMI) Investigator, and by Dr Dimitar Sasselov, who has discovered many exoplanets.

Meet the researcher

Professor Nita Sahai
Fellow of the Mineralogical Society of America
Ohio Research Scholar – Biomaterials
Department of Polymer Science
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Professor Nita Sahai holds the position of Ohio Research Scholar in Biomaterials at the Department of Polymer Science and the Department of Geosciences, University of Akron. She was awarded her PhD degree in 1997 for her work on electrolyte adsorption at the Department of Earth and Planetary Sciences in Johns Hopkins University, Baltimore. As a postdoctoral researcher, she went on to investigate strontium adsorption at oxide and silicate surfaces at Arizona State University, and studied silica and apatite biomineralisation at the Department of Chemistry and Biochemistry, University of Maryland. In 2000, Professor Sahai obtained her first professorship in the Department of Geoscience at the University of Wiscosnin–Madison, where she worked for 11 years before taking her current position at the University of Akron in 2011.
Professor Sahai is a Fellow of the Mineralogical Society of America. Over the course of her varied career, she has been presented with many awards, including Distinguished Lecturer of the Mineralogical Society of America, Romnes Faculty Fellowship – University of Wisconsin–Madison and the NSF CAREER award.

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The University of Akron

SULPHUR METABOLISM ON THE ANAEROBIC EARTH

The mechanisms of sulphur trafficking in present-day methanogens have significant implications for understanding biogeochemical cycles on the primordial Earth. **Dr John Perona** and his team at Portland State University have identified novel proteins essential to both sulphur assimilation and homocysteine biosynthesis, clarifying several important questions in the field.

Life on Earth arose over three billion years ago, in an environment lacking significant quantities of molecular oxygen. As a consequence, the earliest living cells had to rely on distinctive chemical processes that persist today only in extreme conditions where oxygen is still absent. Dr John Perona and his research team at Portland State University in Oregon are studying the genes, enzymes and metabolic pathways for sulphur assimilation in an important class of single-cell anaerobic microbes – or anaerobes – that first evolved on the early Earth. These anaerobes are known as methanogens. They are of particular relevance today because they possess a unique sulphur-dependent metabolism that produces the greenhouse gas methane, a key driver of global warming. Thus, improving our understanding of methanogens could have practical implications for addressing climate change. An enhanced understanding of ancient life might also yield insight into how life could develop on other worlds with alien environments that resemble the early Earth. Finally, Dr Perona and his team expect that a plethora of unique biochemical mechanisms associated with anaerobic sulphur processes await discovery.

Sulphur Metabolism in Methanogens

Early anaerobic life prior to 2.4 billion years ago was capable of carrying out a variety of unique biochemical processes that are suited to an environment lacking oxygen. These biochemical traits are still present in present-day methanogens, a class of microbes that can assimilate sulphur in the form of sulphide. Furthermore, these organisms generate the greenhouse gas methane as a by-product of their energy metabolism, which is consequently known as methanogenesis. Methanogens are probably best known as the hyperthermophilic (heat-loving) organisms, such as *Methanococcus jannaschii,* that inhabit deep-sea hydrothermal vents – hot springs on the ocean floor where life is believed to have originated. These single-celled organisms are classed as 'extremophiles' since they colonise environments that are considered extreme to human beings. Methanogens originated approximately three billion years ago, when Earth's atmosphere was bereft of oxygen, but they persist today in certain places that still lack oxygen, such as at the bottom of the Pacific Ocean, and in the digestive tracts of humans and other animals.

Dr Perona and his colleagues study the metabolic processes in methanogens involving the uptake of sulphur, which exists in the form of sulphide when there is little oxygen in the environment. His work involves discovering new genes and proteins in methanogens that are involved in the unique pathways by which sulphide is assimilated from the environment and then distributed around the cell into proteins, certain RNA molecules, and other metabolites. As he enthusiastically explains, 'the work is very exciting because, from a chemical and biochemical point of view, the early Earth environment is quiet alien to us and may resemble the environment present on some extra-terrestrial planets and satellites. Understanding how early life on Earth was able to arise and adapt may help us recognise the signatures of life elsewhere in the solar system and beyond.' The value of his work has not gone unnoticed, and in 2014, Dr Perona was the recipient of a prestigious research grant from NASA to explore the functions of new proteins in sulphur metabolism.

Dr Perona was initially drawn to this particular field of research due to his passion

‘Understanding how early life on Earth was able to arise and adapt may help us recognise the signatures of life elsewhere in the solar system and beyond.’

for the environment. ‘I’ve had a very general, personal interest in environmental science and environmental protection for a long time, so I was eager to find ways to use my scientific training to make a contribution to solving problems in this area,’ he explains. Given that there is increasing evidence that methane contributes significantly to global warming, Dr Perona’s work is focusing on teasing out novel aspects of methanogen metabolism. ‘These organisms emit all of the biologically derived methane on Earth,’ he adds.

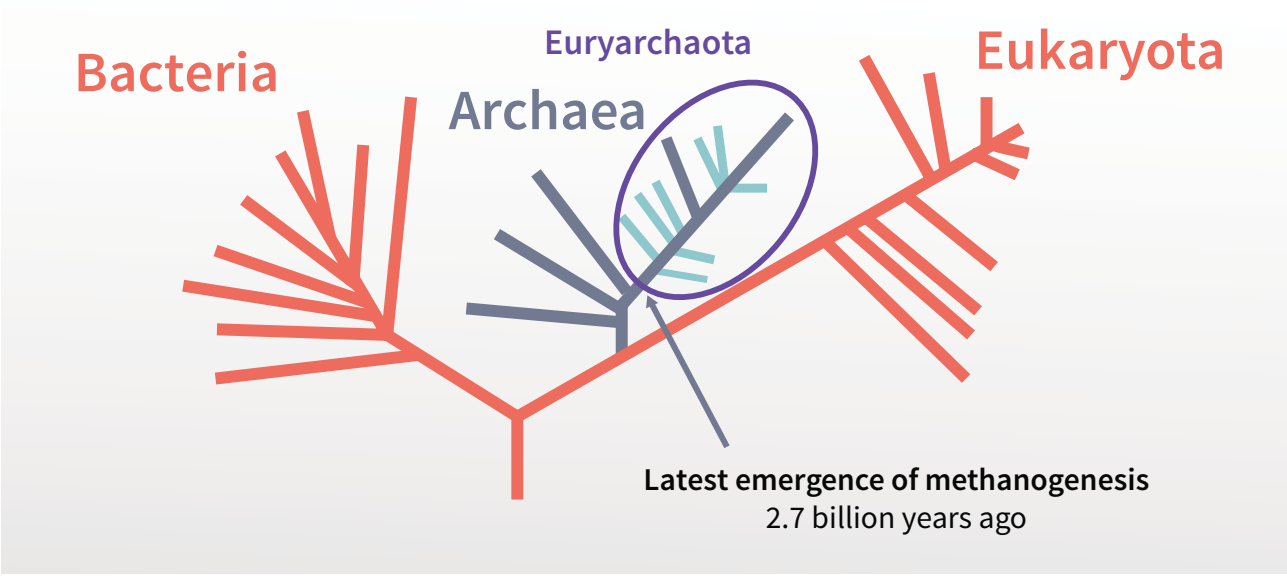
Novel Proteins in Homocysteine Biosynthesis

In order to identify the sulphur assimilation and trafficking pathways in methanogens, Dr Perona and his colleague Dr Benjamin Rauch first performed an exhaustive comparative analysis of the genes present in over 100 single-celled microbes from the domain of life known as the Archaea. Three conserved protein families were discovered that were present in methanogens and closely related organisms, but were always absent from Archaea that thrive in oxygen-rich environments. Intriguingly, the proteins all contained sequence features suggesting involvement in sulphur metabolism, although none had previously been studied or associated with particular molecular processes. Convinced that they were on the right track, the team then used genetic engineering approaches to selectively delete the newly discovered genes from the chromosome of *Methanosarcina acetivorans*, their experimental organism of choice. By comparing the growth characteristics of the natural organism with its engineered counterparts (‘knockout strains’), the researchers were able to demonstrate that two of the new proteins (MA1821 and MA1822) were essential for the biosynthesis of the key amino acid homocysteine (Hcy).

Hcy is very important to the sulphur metabolism of both aerobes and anaerobes, because it is the building block for downstream production of methionine (Met) and S-adenosylmethionine (SAM). Both of these molecules are vital to cellular function. Methionine is an amino acid that is present in all cellular proteins and is consequently essential to all organisms. SAM is a key cofactor molecule that binds to many cellular enzymes to help catalyse reactions that are essential to metabolism. The team’s findings are of great importance, since they filled in a key missing link that was essential to understanding the metabolism of the methanogens. By genome comparisons, it was also found that the key MA1821 gene is present in wide variety of other microbes, all of which also thrive in the absence of oxygen. The work also highlights a distinct contrast with pathways in aerobic bacteria and eukaryotes.

A Novel Route for Hcy Biosynthesis

Dr Perona and his team had made an important breakthrough in assigning a function to two new genes essential to Hcy biosynthesis in *M. acetivorans.* However, they had yet to identify the substrates and mechanisms involved in the biosynthetic pathway. To that end, they teamed up with the laboratory of Dr Bob White at Virginia Polytechnic University to explore the detailed mechanism of how Hcy is biosynthesised in methanogens. This work used cell extracts from the methanogens *M. jannaschii* and *M. acetivorans*, and probed the nature of the pathway using stable isotopically labelled precursor compounds. The team found that



Hcy could be biosynthesised directly from the metabolite aspartate semialdehyde (Asa) – a common intermediate in lysine, threonine, and methionine biosynthesis. Using the previously generated knockout strains, they demonstrated that the MA1821 and MA1822 proteins are essential to catalysing the reaction. These experiments provided the basis for proposing a new enzyme mechanism that, when tested, should expand understanding of sulphur biochemistry generally. These studies carried out by Dr Perona and his colleagues eloquently detailed a novel route for the biosynthesis of homocysteine, while exemplifying unique aspects of sulphur chemistry occurring in primordial life.

Efficient Sulphide Metabolism Requires the MA1715 Protein

Dr Perona and his colleague Dr Benjamin Rauch then addressed the role of the third gene that they discovered by comparing the genomes of methanogens and other Archaea. In *M. acetivorans* this gene encodes the previously unstudied protein MA1715. By comparing the sequence of MA1715 with that of its counterparts – homologues – in other methanogens, they found that the gene accumulated mutations and evolved in the early Earth over timeframes similar to the evolution of the MA1821 protein and indeed to all of the key proteins in the central energy-producing methanogenesis pathway. This was powerful evidence that the newly discovered genes coevolved with methanogenesis and so were likely providing the required sulphur-containing metabolites to that pathway. The newly characterised genes thus formed an essential component of the core metabolic machinery of the ancestral organisms that were among the very earliest life to appear on Earth.

Using the same genetic approaches to delete the gene encoding MA1715, the researchers established that the protein is required for efficient growth when sulphide is used as the sole sulphur source. MA1715 functions like a sulphide biosensor – it allows the cell to take up the compound even when its concentration in the environment is very low. This is clearly a very important characteristic that can enhance the ability of methanogens to survive under adverse conditions. However, the team's further experiments showed that MA1715 might be limited to helping the methanogen cell biosynthesise the sulphur-containing amino acids cysteine, homocysteine and methionine. MA1715 was found not essential for maintaining cellular levels of other important sulphur-containing molecules such as iron-sulphur clusters or certain RNA molecules important to protein synthesis. Therefore, the way in which sulphur is distributed to these and other compounds in the cell remains unknown. Also still to be discovered is the exact biochemical mechanism by which COG2122 enhances sulphide assimilation and helps the sulphur become incorporated into amino acids. Nonetheless, the findings reported by Dr Perona and his colleagues provide the essential framework for going on to solve these important problems.

Sulphur Metabolism – Past meets Future

The fascinating research activities of Dr Perona and his team have revealed some of the intricate and complex mechanisms by which methanogens can metabolise sulphur in oxygen-depleted environments. 'We and others have discovered and are now carefully studying about six new proteins that are unique to the way methanogens assimilate sulphur and distribute it around the cell,' he tells us. 'What we plan next are experiments that address the general question of how life forms transitioned from environments that had no oxygen (like the early Earth) to those that have abundant oxygen (like the Earth today).'

When asked about the future of his team's research, Dr Perona tells us that they 'plan to introduce the new methanogen genes into the common bacterium *Escherichia coli*, which is present in the human gut and is well-suited to serve as a host because it is able to grow both with and without oxygen.' By introducing these methanogen sulphur acquisition genes in various combinations in E. coli, the researchers hope to trace a pathway by which certain sulphur metabolic pathways could have provided evolutionary stepping stones for the transition that occurred when oxygen produced by photosynthesis began to build up in Earth's atmosphere and oceans.

The recent discovery of methanogen sulphur-trafficking proteins genes by Dr Perona and his team open the possibility of discovering novel biochemical mechanisms used by these proteins, a prospect of some significance to our understanding of metabolism generally. Dr Perona's pioneering research provides us with a biochemical appreciation of primordial life on the early Earth and how it has evolved over time, which may ultimately allow us to understand microbial life that could be present on other planets and their moons.

Meet the researcher

Dr John J. Perona
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Portland State University and Oregon Health & Science University
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Dr John Perona completed his PhD in Molecular Biophysics and Biochemistry at Yale University in 1989, where he was trained in protein X-ray crystallography. From 1990–1994, he was a postdoctoral fellow at the University of California, San Francisco, where he employed protein engineering approaches to design and characterize proteases with novel activities. From 1994–2011, Dr Perona served on the faculty of the University of California, Santa Barbara, where he and his co-workers performed detailed structure-function investigations on a variety of nucleic acid enzymes, especially restriction endonucleases and aminoacyl-tRNA synthetases. Dr Perona currently serves as a Professor of Environmental Biochemistry at Portland State University and Adjunct Professor of Biochemistry & Molecular Biology at Oregon Health & Sciences University (OHSU). His research focuses on two areas of biochemistry. First, he and his group are combining genetics and biochemical approaches to investigate how sulphur is assimilated into single-celled microorganisms that inhabit environments without oxygen (anaerobes). The second area of research is devoted to the expansion and modification of the genetic code of bacteria through the use of synthetic biology techniques.

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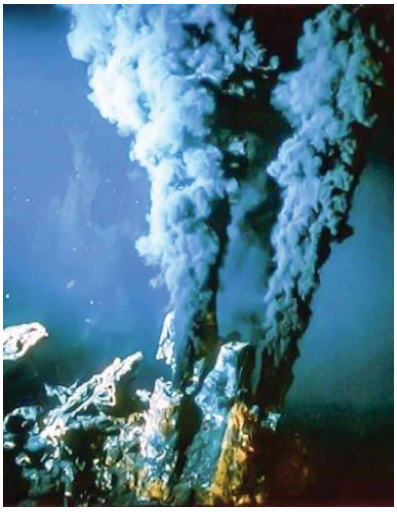
Portland State University

Oregon Health & Science University

UNCOVERING THE RELATIONSHIP BETWEEN AQUATIC THERMOPHILES AND HUMAN BACTERIAL PATHOGENS

Thermophiles thriving on hydrothermal vents are opening new avenues towards understanding the chemical mechanisms of human diseases. Here we discuss the work of **Professors Costantino Vetriani and Frank Robb,** who isolate these extreme microorganisms and study their metabolism, towards the development of novel biotechnological and therapeutic applications.



Active deep-sea hydrothermal vents releasing hot fluids at the bottom of the ocean

Hydrothermal Vents: Teeming with Life

Hydrothermal vents arise as a result of volcanic activity on the seafloor. In volcanically active areas, water enters cracks in the Earth's crust, dissolves minerals, and becomes super-heated by the magma below. This hot water can then erupt as a geyser from a hydrothermal vent, where water temperatures can change from near freezing to 400°C over short distances. The dissolved minerals crash out of the water upon contact with the cold seafloor, gradually leading to the formation of a chimney around the vent.

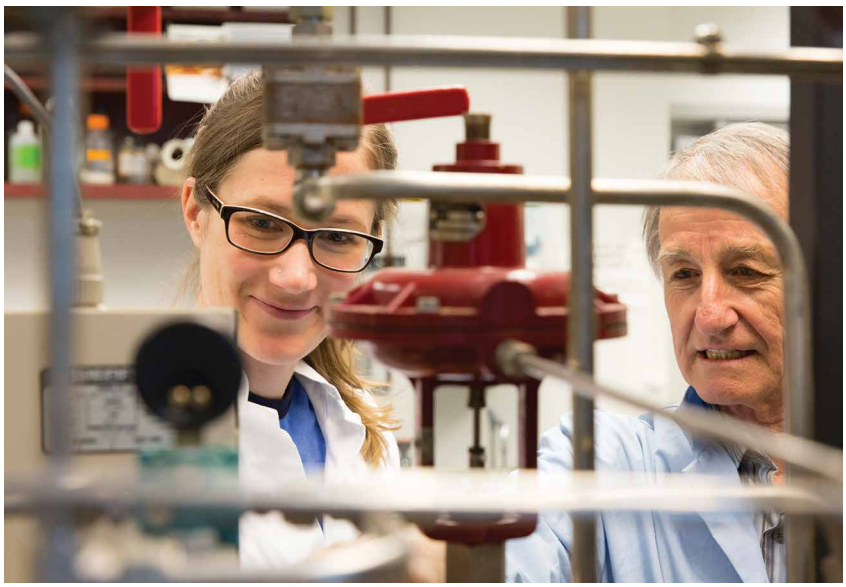
In hydrothermal vent communities, the first step in the food chain are chemosynthetic bacteria – bacteria that extract energy from reactive inorganic compounds such as hydrogen sulphide. These bacteria can exist as either free-living, or in symbiotic relationships with creatures such as vesicomyd clams, vestimentiferan tubeworms, and bathymodiolid mussels. Shrimps that host sulphur-dependent bacteria can also be found near hydrothermal vents.

The microbial communities in these active, hot, venting chimneys have been increasingly studied since the late 1970s. They have attracted many groups of scientists from around the world as research opportunities grow and potential commercial applications are realised.
Here, we discuss the work of two scientists – Professor Costantino Vetriani at Rutgers University and Professor Frank Robb at the University of Maryland – and their contributions to expanding our understanding of the biochemistry of these important marine environments. This pair of scientists first started working together back in 1995, when Professor Vetriani joined Professor Robb's research group as a postdoc, and their collaboration continues to this day.

The Importance of Thermophiles

Hydrothermal systems support an enormous diversity of microbial species that is, in many places, comparable to that of a rainforest. One of the most abundant groups of bacteria are known as *Epsilonproteobacteria.* This group of bacteria include the families *Campylobacteraceae* and *Helicobacteraceae*, represented by the pathogenic *Campylobacter* that can cause gastrointestinal infection in humans and *Helicobacter pylori,* a well-known cause of ulcers in the stomach and duodenum in humans. Disentangling the evolutionary relationships between deep-sea vent *Epsilonproteobacteria* and human pathogens can therefore provide insight into the origins of virulence in pathogens.

Professor Costantino Vetriani is a microbiologist immersed in the ecology and evolution of deep-sea bacteria and the adaptations of these bacteria to extreme environmental conditions. In 2003, Professor Vetriani and colleagues conducted experiments to study the structure and composition of microbial communities in mixtures of cold seawater and fluids heated by magma at hydrothermal vents on the East Pacific Rise – a mid-ocean ridge that runs from the Gulf of California to South of Easter Island in the Pacific Ocean. The aim of their research was to describe the different stages of colonisation of vents by bacterial populations and to uncover any associations between the microbial colonisers and the chemical regimes in the vents. The results were quite interesting – in vents with fluids flowing down the chimneys, the microbial communities were dominated by *Epsilonproteobacteria*, while in parts of the seafloor with no flow, the microbial communities were dominated by *Gammaproteobacteria* related to the family Thiotrichales. Fundamental studies like these have at least two direct applications – they help to improve mathematical models describing the transfer of energy and carbon on the seafloor and to explain the role of microbial communities in transforming the carbon into biomass, which becomes available for higher life-forms.
In fact, several studies have found that *Epsilonproteobacteria* not only dominate communities on the surface of vent chimneys, but they are also common on other animal surfaces. Because of their high biomass, fast growth rates and adaptable



Frank Robb and postdoc Lydia Kreuter operate a specialised high temperature 200L fermenter. CREDIT: Lisa Tossey, University of Delaware

Costantino Vetriani collecting rock colonising microbial biofilms from the gas vents of Tor Caldara, Italy

metabolism, these bacteria are possibly the first to colonise the dynamic diffuse flow vent environments.

Since then, many other studies have shown that these bacteria diverged in the tree of life, as antecedents of their human pathogenic relatives and developed novel systems for respiration, sensing and environmental responses to changes in habitat conditions. Professor Vetriani is also hoping to increase knowledge of bacterial communities in shallow-water (<200 m depth) hydrothermal vents. For many years, these ecosystems were much less studied than deep-sea vents. An important characteristic of the shallow-water vents is that they are largely influenced by photosynthesis. This project took Professor Vetriani and colleagues to Paleochori Bay, on the coast of the Island of Milos, in Greece. Here, the venting area is surrounded by patches of the seagrass *Posidonia oceanica*, where temperatures range from 25 to 119°C. *Epsilonproteobacteria* were again remarkably abundant, representing 60% of the total microbial diversity. Molecular studies revealed the presence of species that had only been previously found at deep-sea hydrothermal vents, such as the chemolithoautotrophic bacterium *Sulfurovum* spp.

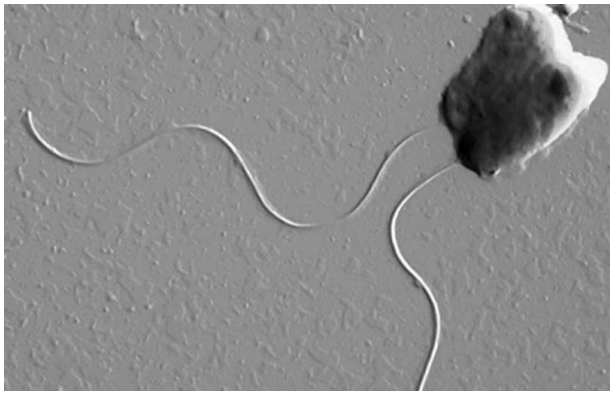
In addition to thriving at high temperatures, certain *Epsilonproteobacteria* from deep-sea vents grow in the absence of oxygen. By culturing them in the laboratory, scientists have found that these bacteria use a diverse spectrum of electron donors and acceptors such as hydrogen, sulphur compounds, nitrate and oxygen for cellular respiration and even share the ability to use the same electron donors and acceptors. For example, *Cetia pacifica*, a new thermophilic bacterium isolated from samples collected from a black smoker chimney on the East Pacific Rise by Professor Vetriani's research team, grew when nitrate or sulphur were available in the growth media. Because of these versatile metabolic characteristics, it is believed that *Epsilonproteobacteria* play a significant role in the ecological and biogeochemical processes of diverse environments on our planet, from marine geothermal habitats to the gastrointestinal tract of mammals.

Linking Chemosynthetic Bacteria to Human Pathogens

Quorum sensing (QS) is a communication mechanism between cells that depends on their density and the production of signalling molecules. QS is used by bacteria to control gene expression, including virulence genes and functions associated with biofilm formation and host colonisation. One QS system that appears to be widespread across the bacterial domain and has been proposed to function as the universal language for communication between species, is based on a furanone derivative known as autoinducer-2 (AI-2) which is synthesised by the LuxS enzyme.

In 2007–2008, Professor Vetriani and colleagues took part in a scientific mission to the East Pacific Rise aboard R/V Atlantis. Using the Deep-Submergence Vehicle Alvin, the researchers used experimental microbial colonisation devices made of stainless steel mesh to sample microbial biofilms. The samples were used in genomic and phylogenetic studies to understand the ancestry of the LuxS lineage and LuxS gene flow in *Epsilonproteobacteria*. The results indicated that LuxS is conserved in all *Epsilonproteobacteria* and that the mesophilic strains, including pathogens, shared a common LuxS ancestor nested within the thermophilic lineage, suggesting that the epsilonproteobacterial LuxS lineage originated in geothermal environments. Further, these analyses indicated two episodes of horizontal gene transfer: *Helicobacter pylori* acquired the gene from *Enterococcus faecium*, a Gram-positive bacterium that also inhabits the human gastrointestinal tract. Moreover, marine *Gammaproteobacteria* of the genera *Photobacterium* and *Vibrio*, along with *Escherichia coli* and other human associated bacteria, share a common LuxS ancestor with *Arcobacter nitrofigilis* and *Arcobacter butzleri*,

A thermophilic microbial mat reveals several layers of anaerobic thermophiles beneath the photosynthetic biofilm covering the consortium



Electron micrograph of Nautilia nitratireducens, a bacterium isolated from a deep-sea hydrothermal vent

a marine organism and a human pathogen, respectively. Together, these results indicated that the LuxS gene represents an evolutionary link between thermophiles and human pathogens.

Chaperones

If early life on earth existed near hydrothermal vents, then heat shock would have been prevalent and life-threatening, as vents frequently ejected hot fluids. In environments with very sharp temperature gradients between hot emissions and ambient water, organisms that could survive the dangerous thermal and chemical conditions close to a vent would be at a huge advantage. There is an important family of proteins produced by cells in response to these stressful conditions – called heat shock proteins (HSPs). Many of them are chaperones, proteins that nurse damaged client proteins back to activity.

These proteins are turned on in response to heat shock to protect the other cellular proteins against denaturation. They work by binding to other proteins during heat shock to prevent them from becoming irreparably damaged by unfolding, and then they help to restore and refold these proteins afterwards.

HSPs are found in all three domains of life – Archaea, Bacteria and Eukarya, and are involved in many vital biochemical processes such as photosynthesis, mitochondrion function, DNA replication, spermatogenesis and membrane function in eukaryotes.

Professor Frank Robb, a microbiologist at the University of Maryland, has been studying these HSPs for many years. His main interest is to learn specifically how thermophiles can stabilise their proteins when exposed to extreme temperatures. The search for thermophiles has taken Professor Robb to some exotic places, including terrestrial and marine volcanic springs.

His working hypothesis is that organisms with well-developed HSPs would have been able to survive closer to vents, where they could grow and reproduce rapidly in the warm water. In contrast, organisms that were safe from sudden heating, far from the vent, would run the opposite risk of finding themselves in cold, barren conditions where they were unable to reproduce.

A specific group of protein aggregates called amyloid fibrils has been linked to more than 15 neurological human diseases, including Alzheimer's and Parkinson's Syndromes. These malfunctions are often called 'conformational disorders', many of which result from the conversion of a normal protein into a specific polymeric amyloid form. In an interesting study conducted by Professor Robb and his colleagues, the mechanism of deconstruction of amyloid fibrils was examined by looking at the disassembly of neurotoxic fibrils by chaperones both in the presence and absence of ATP – the vital molecule that transports chemical energy within cells.

This study indicated that HSPs adsorbed on the fibril surface formed clusters with no ATP present, at specific intervals on the fibril surface where these chaperones could unravel the fibril to release short fragments. Their motivation for using HSPs to disintegrate amyloid fibrils is based on the potential of these proteins to restore the form of native ones and normal biological activity. From these studies, Professor Robb and his team demonstrated a way to disperse fibrils with minimal increase in cytotoxicity.

In a previous study, the team had constructed a cold-adapted mutant form of HSP60 from the hyperthermophile *Pyrococcus furiosus*, a bacterium that grows at temperatures of around 100°C. This organism had been isolated from the shallow marine volcanic hydrothermal vent systems. The results presented in this study were considered a potential advance towards the development of a treatment for protein conformation diseases in humans.

Other applications of Professor Robb's current studies are methods to improve the durability and immunogenicity of vaccines through the expression of HSPs from thermophiles. He and his team are hoping to identify molecular processes that could enable the long-term storage of vaccines. This would be an important step forward for the supply of vaccines to remote locations that lack refrigeration, such as developing countries.

There is growing excitement in the field of thermophile molecular biology. Hydrothermal vents offer ideal conditions for the evolution of microbial metabolic processes. The evolutionary links between human and animal pathogens and their non-pathogenic deep-sea relatives studied by Professor Vetriani have revealed the role of genomic plasticity in the diversification of microbial life. Thermophiles are also model systems to study complex cellular functions relevant to human disease, such as protein folding and stress responses. Studies by Professor Robb on HSPs in thermophiles provide a better understanding of the progressive neurodegenerative stages in Alzheimer's and Parkinson's, making these proteins potential therapeutic candidates.

CREDIT: Nerissa Escanlar

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Professor Costantino Vetriani completed a MSc in Microbiology at the University of Rome La Sapienza and a PhD in Molecular Biology in 1994 at the University of Rome Tor Vergata. Before joining Rutgers University in 1997, Dr Vetriani was a guest investigator at Woods Hole Oceanographic Institution and Research Associate at the Center of Marine Biotechnology in Baltimore in Professor Robb's laboratory. Research in his laboratory focuses on the ecology and evolutionary relationships of deep-sea prokaryotes and the microbial adaptations to extreme environmental conditions. He has published more than 60 peer reviewed papers and has participated in 20 oceanographic expeditions.

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PINNING DOWN THE HABITABLE ZONES OF DIFFERENT STARS

One of life's greatest mysteries is whether or not we are alone in the Universe. One way to find planets that could support life is by working out whether they lie in the 'habitable zone' of their parent star – a distance at which liquid water might exist on the surface. **Professor Jim Kasting** at Penn State University has been studying stars and the boundaries of their habitable zones for decades. More recently, he has been looking into how these boundaries can change with a phenomenon called climate limit cycling, which might occur on certain planets.

The Habitable Zone

Finding extra-terrestrial planets that may be able to support life is one of the biggest goals in astronomy. Since life as we know it relies on water to survive, one of the requirements for finding potentially habitable worlds is that liquid water must be able to exist on the planets' surface. Life could exist on planets with subsurface water, as well, but it would be difficult to detect remotely, and so is of less interest to astronomers.

A planet too close to its star would be too hot, so any liquid water would evaporate, while a planet too far away would be too cold, and water would freeze. Astronomers have come up with the term 'habitable zone' to describe the area around a star that would be 'just right' for liquid water to exist. This region is also often called the 'Goldilocks zone' since, like the porridge in the well-known tale, it has to be just right.

Water is thought to be key in finding life, because all life as we know it relies on liquid water to exist. However, just because liquid water might be present on a planet does not mean humans could survive there, but astronomers consider the existence of water essential for Earth-like life-forms to survive.

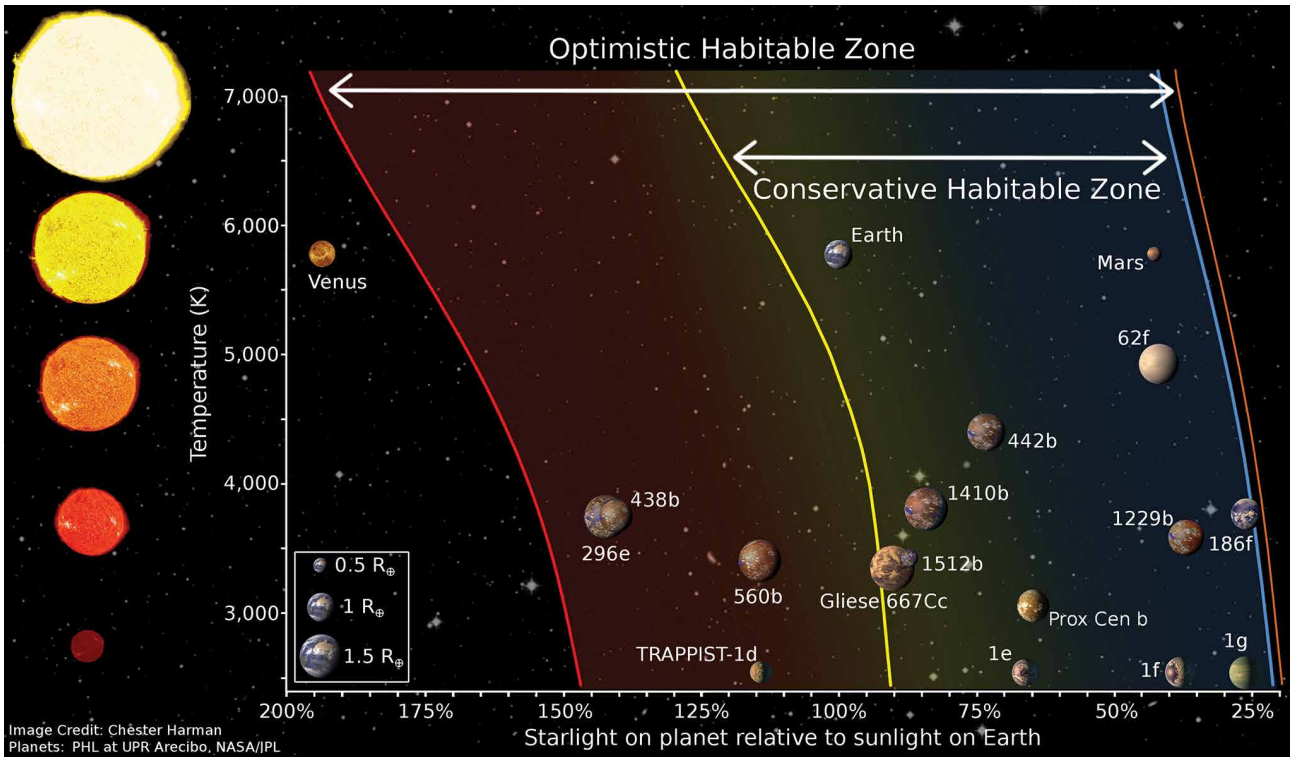
Identifying terrestrial planets in the habitable zones of other stars is one of the primary goals of ongoing space surveys and proposed future space missions. NASA's Kepler Space Telescope has so far detected over 3,000 planet candidates, about twelve of which are most likely rocky planets within the habitable zones of their host stars. But exactly how we know whether or not water can exist on a planet is a complicated question, with a rich history.

Over twenty years ago, in 1993, Professor Jim Kasting and his colleagues came up with a model to place restrictions on where the habitable zone could be for certain planets. 'I derived estimates for the boundaries of the liquid water habitable zone using a 1-D climate model,' explains Professor Kasting. The team came up with a set of inner and outer boundaries for habitable zones, as a function of the host star's size and distance.

They defined the boundary's inner edge as the point where water would be lost through photolysis – breaking up into hydrogen and oxygen – driven by light from the Sun. The outer edge was determined as the point where even a maximum amount of carbon dioxide – a greenhouse gas – fails to keep the surface of a planet above freezing temperatures. Their model showed that the habitable zone of our Solar System is located between 0.95 and 1.67 AU from the Sun (1 AU, or 'astronomical unit', refers to the average distance between Earth and the Sun – about 150 million kilometres!). This model was used in most calculations for a long time, without being updated.

Twenty years later, Professor Kasting updated the model with new estimates. The calculations were done by a team of researchers, including his then postdoc Ravi

‘Climate limit cycles might apply to our own Solar System planet Mars during its early history. Early Mars is slightly beyond the outer edge of the habitable zone, so we need H_2, in addition to CO_2 and H_2O, to warm it up.’



Kopparapu and graduate student Ramses Ramirez. The updated paper, published in 2013, included more accurate calculations for factors such as the amount of energy that water vapour and carbon dioxide absorb. It also included the effect of light scattering by water vapour. ‘That moved the inner edge out from 0.95 AU to almost 1.00 AU, that is, close to Earth’s orbit,’ he says.

The problem here, was that having a boundary so close to Earth meant that we live on the edge of being non-existent. But this was based on a one-dimensional model, and could be further improved. ‘Fortunately, a French group came along and re-did the inner edge calculation using a 3-D climate model,’ Professor Kasting explains. ‘That moved the inner edge back to 0.95 AU, where it had been previously.’ The study, published in *Nature* in 2013, was carried out by Jeremy Leconte and colleagues from Laboratoire de Meteorologie Dynamique in Paris and the University of Chicago.

The three-dimensional model solved many issues. Before then, the habitable zone had mostly been studied using one-dimensional atmospheric models. As a consequence, the mechanism relied on the assumption that the troposphere is saturated in water vapour, and did not account for differences in cloud distribution. 3-D climate models are also needed to study planets around red dwarf (or ‘M’) stars. Because these stars are so dim, any potentially habitable planet must orbit quite close to the star; thus, it could become tidally locked, only showing one face to the star, as the Moon does to the Earth. 1-D models do not do a good job in this situation.

However, despite these improvements, there were still some effects that were yet to be taken into account. This would again move the goal posts of habitable zones.

Climate Limit Cycling

Climate limit cycling happens when a planet undergoes alternating warm and globally glaciated periods, rather than having stable warm climates. ‘An astronomer at University of Toronto showed that some planets near the outer edge of the habitable zone might experience climate limit cycling,’ Professor Kasting tells Scientia. The astronomer was Kristen Menou, who published a paper in the journal *Earth and Planetary Science Letters*, outlining how climate limit cycling could affect a planet’s habitable zone. He explained how Earth-like planets receiving less sunlight than our Earth may no longer possess a stable warm climate, but instead repeatedly cycle between unstable glaciated and de-glaciated climatic states.

At temperatures above freezing, rocks can be weathered (dissolved), and they weather faster at higher temperatures. Carbon dioxide plays a role in this weathering, and is taken up during the process. This intake reduces the concentration of carbon dioxide in the atmosphere, and due to the resulting reduction in the greenhouse effect, the climate cools, ultimately leading to freezing conditions. On a frozen planet where no weathering takes place, carbon dioxide builds up in the atmosphere from the continued release of gas from volcanoes. This warms up the planet until liquid water can exist on the surface, and weathering starts to take place once again. This cycle could have implications for the search for life on exoplanets in the habitable zone of nearby stars.


CREDIT: NASA/JPL-Caltech

Following the publication of this paper, Professor Kasting and colleagues took another look at the impact climate cycling would have on habitable zones. 'We repeated those calculations, correcting an error in the volcanic outgassing rate, and showed that this limit cycling activity was less prevalent than Menou had found,' he explains.

Professor Kasting and his colleagues argued that an abiotic Earth would have a greater carbon dioxide concentration than today, because plants and other biota help to pump up the carbon dioxide concentration in soil. When they tuned their abiotic model accordingly, they found limit cycles could occur, but that previous calculations had overestimated their importance.

Their study found that Earth-like planets with volcanic outgassing rates similar to those of today can maintain stable climates across the entire range of the habitable zone, regardless of the type of star they orbit. The type of host star, along with weathering and volcanic activity, turned out to all be important factors in determining whether a planet would be prone to limit cycling. The team also identified the best kind of stars for planets to orbit, in terms of their chances at being habitable.

Planets around F-stars – those between 1 and 1.4 times the mass of the Sun – were found to be the most prone to limit cycling behaviour, as a result of their susceptibility to ice-albedo feedback. This means they are likely to experience punctuated episodes of warm conditions followed by extended glacial periods.

The team also found that planets orbiting late K and M stars, which tend to be quite a bit smaller than our Sun, avoid limit cycles because of reduced ice-albedo feedback. However, these planets may suffer from water loss during their formation. The team concluded that systems with the greatest potential for habitability are those around late G- and early K-type stars, which are between 0.8 and 1.2 the mass of our Sun.

Early Mars

In 2014, Professor Kasting and his colleagues applied the concept of climate limit cycling to models of the early Solar System, and discovered that young Mars might have been affected by the same kind of changes. 'This same type of limit cycling might apply to our own Solar System planet Mars during its early history,' he explains. Climate-driven changes in the temperature of young Mars bring it into the habitable zone, meaning it could have once supported life.

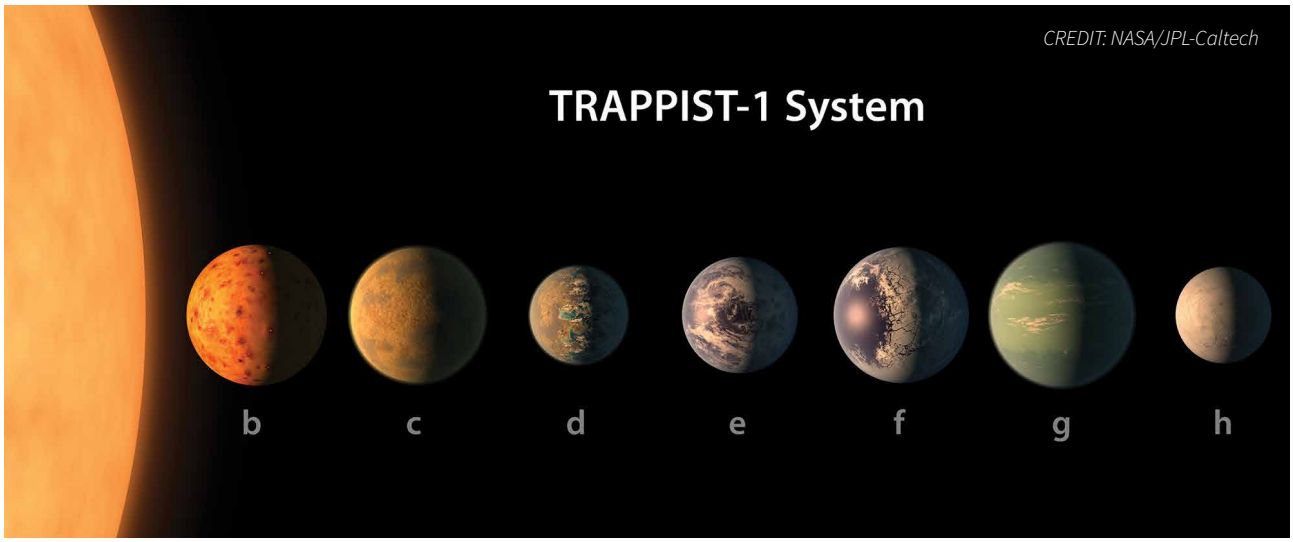
We know from studying Mars that it once held vast amounts of liquid water. Marks on its surface suggest the presence of an ocean, with some theories suggesting that it covered a third of the planet. For example, orbiters as far back as 1976 discovered two possible ancient shorelines near the pole – named Arabia and Deuteronilus – each thousands of kilometres long. Evidence has also been found in the chemical properties of Mars' soil and atmosphere. A 2016 study carried out by NASA suggested that the volume of Mars' early ocean was at least 20 million cubic kilometres, which is more than Earth's Arctic Ocean.

Young Mars, however, was slightly too far away from the Sun for liquid water, according to Professor Kasting. 'Early Mars is slightly beyond the outer edge of the habitable zone, so we need H_2, in addition to CO_2 and H_2O, to warm it up,' he explains. In order for liquid water to remain on the surface, early Mars would have needed a denser atmosphere, along with a warmer climate.

Signatures of Life

In a 2014 paper published in the Proceedings of the National Academy of Sciences (PNAS) Professor Kasting and his colleagues provided a review of habitable zones and remote biosignatures – or signs of life. In this paper, they outlined habitable zone boundaries along with the criteria needed to detect life remotely, and predicted the frequency of Earth-like habitable planets orbiting M and K type stars.

‘The James Webb Space Telescope may observe some transiting Earth-sized planets around M stars starting as early as next year.’



The team found that the conditions needed for liquid water on a planet continue to divide researchers. ‘Exactly what conditions are needed to maintain liquid water remains a topic for debate,’ they wrote in the paper. As with early Mars, adding H_2 to a planet’s atmosphere can warm the planet and move the outer edge of the habitable zone farther out. Conversely (and counter-intuitively), planets with less water than Earth might actually be habitable closer to their parent star because the climate feedback caused by water vapour would be weaker.

Professor Kasting and his colleagues also came up with new guidelines to help scientists building the next generation of space telescopes, based on the detection of biosignatures. These telescopes will use so-called ‘direct imaging’ to look at planets around all the nearby stars. Such a telescope would use a coronagraph or a starshade to block out the light from the star, while retaining the reflected light from the planets around it. Doing so will enable astronomers to perform detailed spectroscopy, that is, looking at the frequencies (or colours) of light that are absorbed by the planet’s atmosphere, which tell scientists what gases are present.

The most interesting compounds to look for are called biomarker gases: species that might indicate whether life is present on a planet. One obvious choice is molecular oxygen, O_2, which makes up 21% of Earth’s atmosphere. Most of Earth’s O_2 is produced by photosynthesis, and thus, is a direct by-product of life. But O_2 can also be produced by some abiotic processes, for example, by photodissociation (splitting apart) of water vapour, followed by the escape of hydrogen to space. The key to identifying life is to simultaneously look for reduced gases such as methane, CH_4, or nitrous oxide, N_2O. These gases, which are also produced by organisms, react with O_2 and should not be found if life is not also present.

Future Space Telescopes

Now that we know where to look and what to look for, the next generation of observing technology will allow us to search for extra-terrestrial life like never before. Professor Kasting says the future of space telescopes will provide the next step in studying planets in the habitable zone.

In particular, he points out NASA’s HabEx and LUVOIR projects – both being developed in the hopes of observing these planets within the next 20 years. These projects – the Habitable Exoplanet Imaging Mission (HabEx) and the Large UV/Optical/IR Surveyor (LUVOIR), are only concepts at the moment. They have both been selected for further study until 2020, when decisions will be made about whether or not to carry them out.

HabEx’s main goal is to directly image Earth-like exoplanets, and measure the composition of their atmospheres, for the first time. LUVOIR is a larger, general purpose space telescope that could also study a variety of exoplanets, including potentially habitable ones. Both of these projects would put Professor Kasting’s life work to the test in a practical way, by studying planets that lie within the habitable zones of their host stars.

Long before these concepts become reality, however, another mission will begin to apply our understanding of habitable zones. NASA’s James Webb Space Telescope (JWST) will start to search for life almost as soon as it is launched, which is planned for October next year.

The telescope is set to be the largest space telescope yet, and NASA hopes to use it to study everything from nearby stars to the most distant visible Universe. This will, of course, include looking at potentially habitable planets. Unlike HabEx and LUVOIR, JWST will only be able to look at planets that transit (pass in front of) their parent star. Most planets should not do this because their orbits are not aligned with our line of sight from Earth. But we already know of a few potentially habitable planets that do transit their star and that are not too far away. Three of the planets orbiting the M star TRAPPIST-1, 40 light years away, fall into this category. Thus, if we are lucky, we could detect signs of life on other planets as early as next year.

Meet the researcher

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Since 1994, Professor Jim Kasting has been a Professor of Geosciences and Meteorology at Penn State University, where he has been an Evan Pugh University Professor since 2012. After obtaining his AB in Chemistry and Physics at Harvard University in 1975, Professor Kasting went on to receive a PhD in Atmospheric Science at the University of Michigan in 1979. From 1979–1981, he worked for the National Center for Atmospheric Research. Then, between 1981 and 1988, he was first a postdoc, then a research scientist at NASA's Ames Research Center, in the Space Science Division. In 1988, he became an Associate Professor at Penn State, before becoming Professor in 1994. Between 2003 and 2004, he was a visiting professor at NASA's Jet Propulsion Laboratory and in 2006, he also spent time at Laboratoire des Sciences du Climat et de l'Environment in Gif-sur-Yvette, France.

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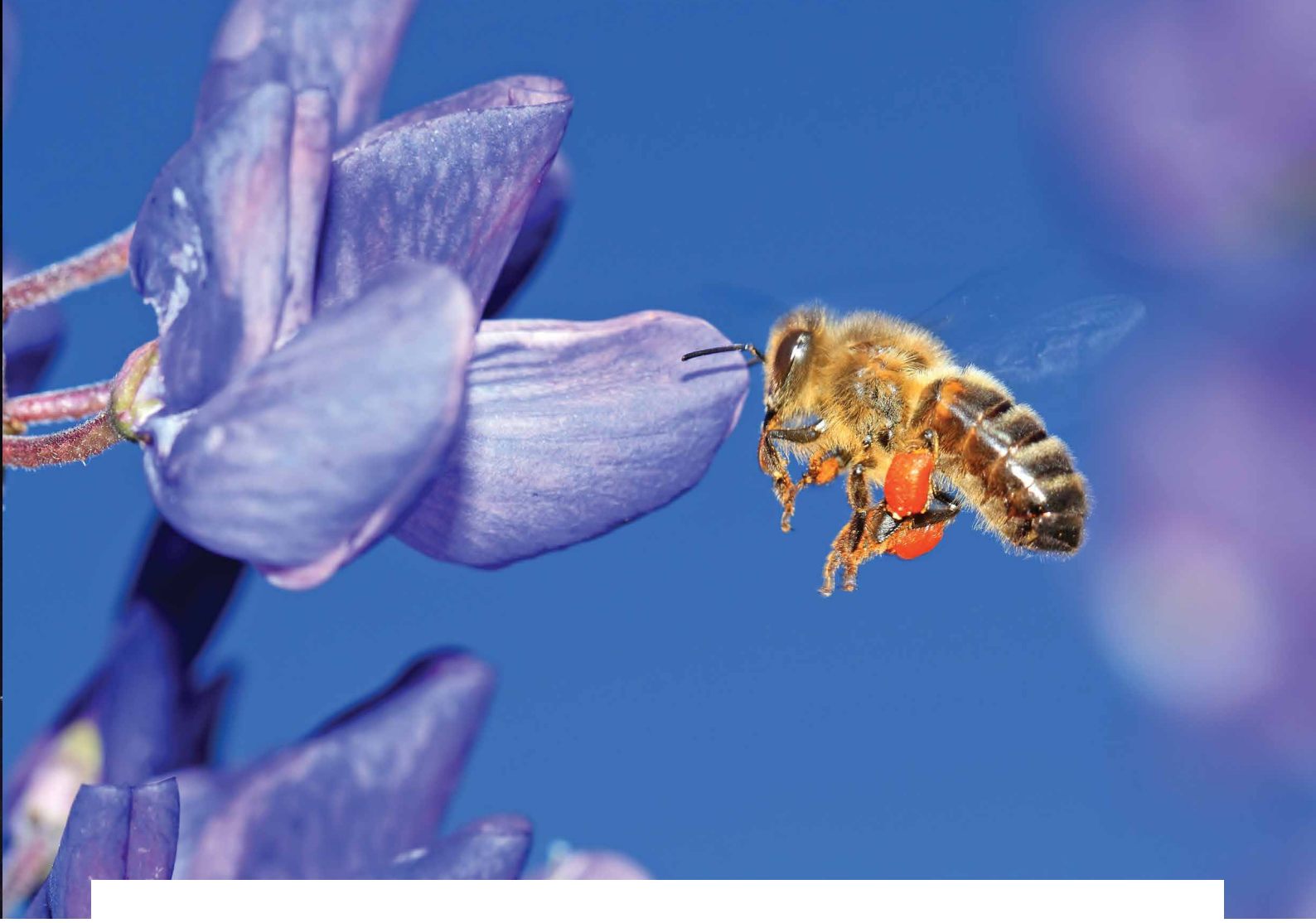


CELEBRATING OUR PLANET'S BIODIVERSITY

Since life first emerged on Earth, within the habitable zone of our parent star, it has flourished into a multitude of forms. Evolution has furnished our planet with a bewildering array of organisms, from bacteria to birds, and from primroses to primates. Life on other planets has not yet been found, and so far, this is the only show in town, so we had better protect it while we still can. In this section of the issue we explore some of the amazing lifeforms we share our world with, and some of the threats they face, such as climate change and pollution.

Indeed, our rapidly changing climate has already begun to leave its mark on lifeforms in surprising ways. Dr Suzie Currie of Mount Allison University in Canada investigates the effect of anthropogenic climate change on marine creatures, specifically fish. As conditions in the seas change drastically, fish will need to adapt or perish. Dr Currie investigates their ability to do just that, and has found that both physiological and social strategies play key roles. Also investigating how organisms adapt to changes in climate is Dr Branwen Williams at the Claremont Colleges in California, who studies the effect of climate change on coralline red algae found in the Arctic-Subarctic. These algal lifeforms live for up to 850 years, and store records of the ocean's conditions as they grow, including information about pH, salinity and temperature. Therefore, Dr Williams and her team believe that these algal life forms are recording climate change as we speak.

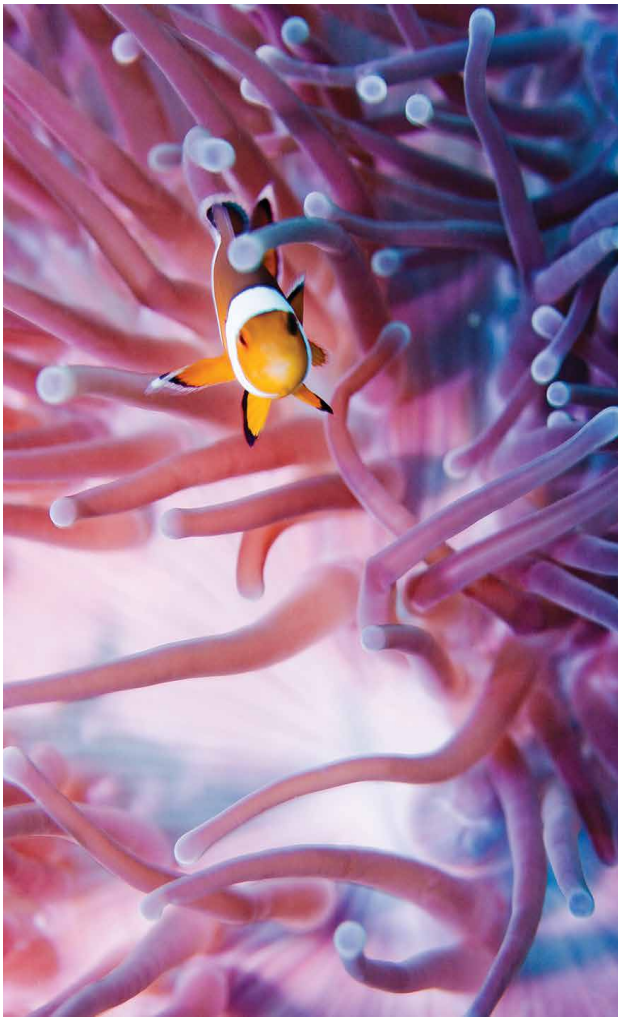
Of course, human-induced climate change is not the only danger that organisms on this planet face. Good old-fashioned pollution also represents an existential threat to countless lifeforms on Earth. Dr Reinhard Dallinger and his colleagues at the University of Innsbruck in Austria investigate levels of metals in invertebrates, as a way to measure metal pollution in the environment. By exploring metal metabolism, regulation and detoxification in various invertebrates, the team can locate and measure environmental metal fluxes and pollution.

This section also showcases research into diverse animal behaviours. First, we highlight the work of Dr Mollie Manier and her team at The George Washington University. Her research has revealed how female fruit flies

choose the best quality sperm to fertilise their eggs, after mating with multiple males. These flies boast among the longest documented sperm cells, at a staggering 5.8 cm long, which is 20 times the length of the fly itself, and approximately 1000 times the length of a human sperm cell. After mating with multiple males, female flies are able to give sperm from certain males a greater chance at fertilisation.

Next, we explore the remarkable lives of prairie dogs, and they aren't as cuddly as you would think. Dr John Hoogland, of the University of Maryland, has studied prairie dogs in the United States for the past 44 years, in which time he has made countless discoveries about these remarkable rodents. In one surprising observation, his team found that these animals commit infanticide under certain circumstances, by killing each other's litters. However, this is just one behaviour in a huge variety that Dr Hoogland has recorded. So too, for Dr Mirjam Knörnschild and her group at the Free University Berlin. However, Dr Knörnschild primarily studies the acoustic communication and social behaviours of wild bats. Her research group studies how bats communicate through song, with the aim of understanding the interplay of social and vocal complexity in culturally transmitted song dialects.

The diversity of life on Earth, some of which is showcased here, is under threat. We end this section with an interview with Jenny Vidler and Sharon Johnson of the Galapagos Conservation Trust (GCT), who discuss some of the Galapagos' iconic species that are currently threatened, due to human activity. GCT is the only UK registered charity to focus exclusively on the conservation and sustainability of the Galapagos Archipelago. Their mission is to support, develop and promote projects that achieve measurable conservation, sustainable living, and protection of the environment.



COPING WITH STRESS IN AN AQUATIC WORLD

As high temperatures continue to set records around the globe, aquatic organisms are facing new environmental extremes. Freshwater and marine animals will either adapt to resulting changes in salinity, temperature and oxygen, or face mortality and possible extinction. Scientists such as **Professor Suzie Currie** are investigating the inherent plasticity – or flexibility – of fish to cope with stressful conditions. She and her team at Mount Allison University in Canada are discovering that both physiological and social strategies may affect how fish cope with their new environmental realities.

Estuaries – Models of Extreme Conditions

Professor Suzie Currie's interest in how fish species will fair with climate change began in her undergraduate student years. 'I became interested in how animals function, particularly in extreme or stressful environments,' she explains. 'Having grown up on the east coast of Canada, I am also very drawn to the water, so it made sense for me to combine my love of the ocean with my interest in animal function and use fish as a model to understand how environmental change affects animal functioning.'

One ideal place to study how fish already function in environmental extremes is an estuary. Estuaries are enclosed bodies of water that have a connection to the ocean and receive freshwater flow. As such, these bodies of water can vary in salinity ranging from little or no measurable salt content (such as freshwater) to that found in sea water (35 parts of salt per thousand parts of water), or even higher.

Many estuarine fish are somewhat adapted to slight variations in salinity, but extreme events associated with climate change might pose another problem. For example, heavy rainfall or prolonged drought can more rapidly and radically alter an estuary's salinity, perhaps beyond the limitations of its inhabitants. Therefore, a key question for Professor Currie and her research group at Mount Allison University is how flexible – or plastic – fish already are in responding to these types of events.

In two 2016 studies, Professor Currie's team collaborated with Dr Jayson Semmens of the Institute for Marine and Antarctic Studies in Australia to work out how low salt (hyposaline) and high salt (hypersaline) conditions affect two different shark species. Their study targets were gummy sharks *(Mustelus antarcticus)* and school sharks *(Galeorhinus galeus)*, which both use the Pittwater Estuary located in southeast Tasmania, Australia. Like many estuaries, the Pittwater is an important refuge and nursery for many kinds of sharks and fish because it is shallow, full of food and protected.

Depending on the weather and how much freshwater and seawater flows in, the Pittwater experiences large fluctuations in salinity. During wetter summers, increased rainfall dilutes the estuary and causes hyposalinity, dropping concentrations to

‘The long-term objective of my research program is to understand the feedback between physiological and behavioural responses allowing animals to compensate and maintain function in changing, often stressful environments’

25.5–28 parts per thousand. Conversely, dry summers increase evaporation in the Pittwater and cause hypersalinity, reaching between 40 and 47 parts per thousand. Previous studies suggested that sharks migrate in or out depending on wet or dry summer conditions.

The researchers aimed to replicate these dilute or concentrated-like conditions in a controlled laboratory setting. They took juvenile gummy and school sharks from the wild and let them acclimatise in large outdoor seawater tanks. They then subjected them to both low and high salinity levels for 48 hours. Blood and tissues were then analysed for all sorts of physiological parameters, including red blood cell counts, haemoglobin, electrolytes, proteins, ubiquitin, osmolytes and oxygen consumption, among others indicators of stress.

During low salinity conditions – also known as hyposmotic events – the researchers found that gummy sharks did not fare as well as school sharks. Likewise, hypersaline conditions generally caused more stress in gummy sharks.

Evolutionary adaptation has probably made the school shark more resistant to changing salinities. The physiological differences between the two sharks explain why the gummy shark is less numerous in Pittwater Estuary, especially after heavy rainfall or dry spells. ‘We have shown that these changes in salinity result in species-specific physiological stress and our work provides physiological correlates to the observed distribution patterns and movement of shark species in their critical nursery grounds,’ Professor Currie tells us.

Rising Temperatures in Salmon Rivers

Along with sharks, Professor Currie’s group is also looking at how extreme weather might affect Atlantic salmon *(Salmo salar).* The number of these salmon returning to spawn in the Miramichi River in eastern Canada is dropping to all time historic lows. The trend is alarming, since more than 20% of the total North American population of these fish come from there. High water temperatures appear to be the cause. ‘Thermal stress is a major concern for Atlantic salmon populations, with mortalities observed after several days of high summer temperatures,’ explains Professor Currie. Ideally, water temperature for Atlantic salmon should be between 6–20°C. In the summers of 2014 and 2015, however, temperatures in the Miramichi averaged 21°C, with some peak temperatures recorded as high as 27°C.

The suspicious culprit is likely changing climate, says Professor Currie: ‘High temperatures and low water levels associated with climate change are hypothesised to be key contributing factors in this decline. In addition, temperature changes are frequently quite rapid, allowing little time for fish to acclimatise or recover from such stressful conditions.’

Professor Currie explains that as of now, ‘many of the physiological and cellular mechanisms underlying thermal stress in Atlantic salmon are poorly understood.’ Previous laboratory studies tended to focus on the effects of constant, and not fluctuating, temperatures on salmon health. However, in the Miramichi River, as in most aquatic environments, temperatures rise and fall on a daily basis. Does this natural diel cycle physiologically harden salmon for stress? Does it afford them more plasticity to adapt to higher temperatures?

To find out, Professor Currie, her collaborator, Dr Tyson MacCormack and their graduate student, Louise Tunnah, set out to replicate more real-world conditions in their laboratory design. After acclimatising young Miramichi salmon in large tanks, they subjected them to daily temperature fluctuations common on the river. After three days, they exposed the salmon to thermal shock. To evaluate stress, they measured parameters such as oxygen consumption, metabolic rate, blood cell counts and protein damage in the young salmon.

They found that salmon that had undergone the daily temperature changes were not hardened to stress. On the contrary, these salmon had notable physiological and cellular responses to fluctuating temperatures. 'We have been using real-world field data to replicate these thermal conditions in the lab, subjecting salmon to natural diel warming cycles. Notably, most studies that investigate the effects of temperature on animals acclimate animals to a constant temperature. Our data indicate that thermal cycling significantly affects several physiological responses,' Professor Currie summarises.

Better Together

The team's shark and salmon studies demonstrate that changes in physical parameters such as salinity and temperature cause stress at the physiological and cellular levels. But Professor Currie is also delving into how other features such as social environment play a role. 'My students and I study a variety of fish species (for example, salmon, trout, tropical mangrove fish, sharks) and determine if they are negatively affected by realistic environmental change,' she tells Scientia. 'We also ask whether or not the social environment (for example, isolation, pairs, and groups) influences how they respond to their environment.'

As an example, Professor Currie points to social behaviour of rainbow trout *(Oncorhynchus mykiss)* and their responses to thermal stress. When resources become limited in the wild, juvenile rainbow trout are known to form social hierarchies whereby some fish dominate food and shelter over weaker fish.

Since trout behave similarly in laboratory tanks, Professor Currie along with her colleagues and then graduate student, Sacha LeBlanc, were able to conduct controlled experiments on how these already-socially stressed fish respond to increases in water temperatures. After applying thermal stress, the researchers measured the amount of stress hormone and heat shock proteins in tissue and blood samples of both dominant and subordinate fish.

Their studies found that subordinate trout were significantly less tolerant of heat. Specifically, thermal stress induced higher levels of heat shock proteins (HSPs) in the weaker fish, suggesting that social environment is having an effect on fish at a cellular level. However, the mechanism by which this is happening is unknown. 'This is a gap in our knowledge and one we are currently pursuing,' explains Professor Currie. 'Key to this research theme is the ability to distinguish the influences of genetic and environmental variability and the interaction between the two.'

To further understand environmental plasticity, the team is looking at the mangrove rivulus (*Kryptolebias marmoratus*), a fish found in mangroves in and around the Caribbean and Central America. The fact that this fish is an air breather suggests that it has evolved to live in extreme and variable conditions. The rivulus is also the only known vertebrate hermaphrodite, meaning it has both male and female traits and essentially produces 'clones' of itself. As such, the genetic uniformity of rivulus makes it an ideal model. 'We can use this fish to isolate genetics and examine contributions of environmental variation on behavioural and physiological traits,' says Professor Currie. 'We are using mangrove rivulus to understand the flexibility or plasticity of traits involved in thermal and salinity tolerance and how changes in fish behaviour may impact the responses to environmental stress.'

Even though rivulus fish can reproduce on their own, it looks like they might be better off when they have other rivulus around to stay healthy, especially in extreme conditions. Professor Currie's preliminary data suggests that when they become socially isolated, rivulus fail to produce heat shock proteins that protect their cells from being damaged by thermal stress. As it turns out, social living is a plus.

The team has extended their research with rainbow trout and mangrove rivulus to Atlantic salmon. They are currently studying whether its schooling behaviour affects their metabolism and swimming.

Intersection of Physiological and Social Worlds

Professor Currie emphasises that scientists need to consider both social aspects and biological responses when understanding how an animal might cope with climate change. She tells Scientia: 'Our work has broad physiological significance as well as enhancing our understanding of fish stress biology. Understanding where the social and physical environments intersect is important in understanding how animals cope with stress and what conditions make animals more susceptible to stress.'

She believes fish are useful models to make these connections. By identifying and studying their stress-coping strategies, we 'may uncover unique and effective mechanisms used by fish in their natural environment that may inform physiological studies on mammals and humans,' she says. Also, identifying their vulnerabilities to climate change will help decisions on how to manage or restore their habitats.

Professor Currie's group will pursue studies on the social-physical environment 'intersection' by conducting further experiments in both laboratory and field settings. 'My programme will also continue to focus on the importance of ecologically realistic environments in studying physiological responses of animals,' she adds.

Meet the researcher

Professor Suzie Currie
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Professor Suzie Currie is a comparative animal physiologist and professor at Mount Allison University in New Brunswick, Canada, where she also currently heads the Department of Biology. She received her BSc in Honours Biology at Acadia University in Nova Scotia and then completed a MSc in 1993 and PhD in 1997, both in Biology from Queen's University in Ontario. She conducted postdoctoral research until 2000 at the Department of Zoology at Cambridge University, where she was the Charles and Katherine Darwin Research Fellow at Darwin College. Professor Currie teaches and advises many undergraduates and graduates in biology and animal physiology. Her research focuses on understanding the behavioural and physiological responses of animals in changing environments.

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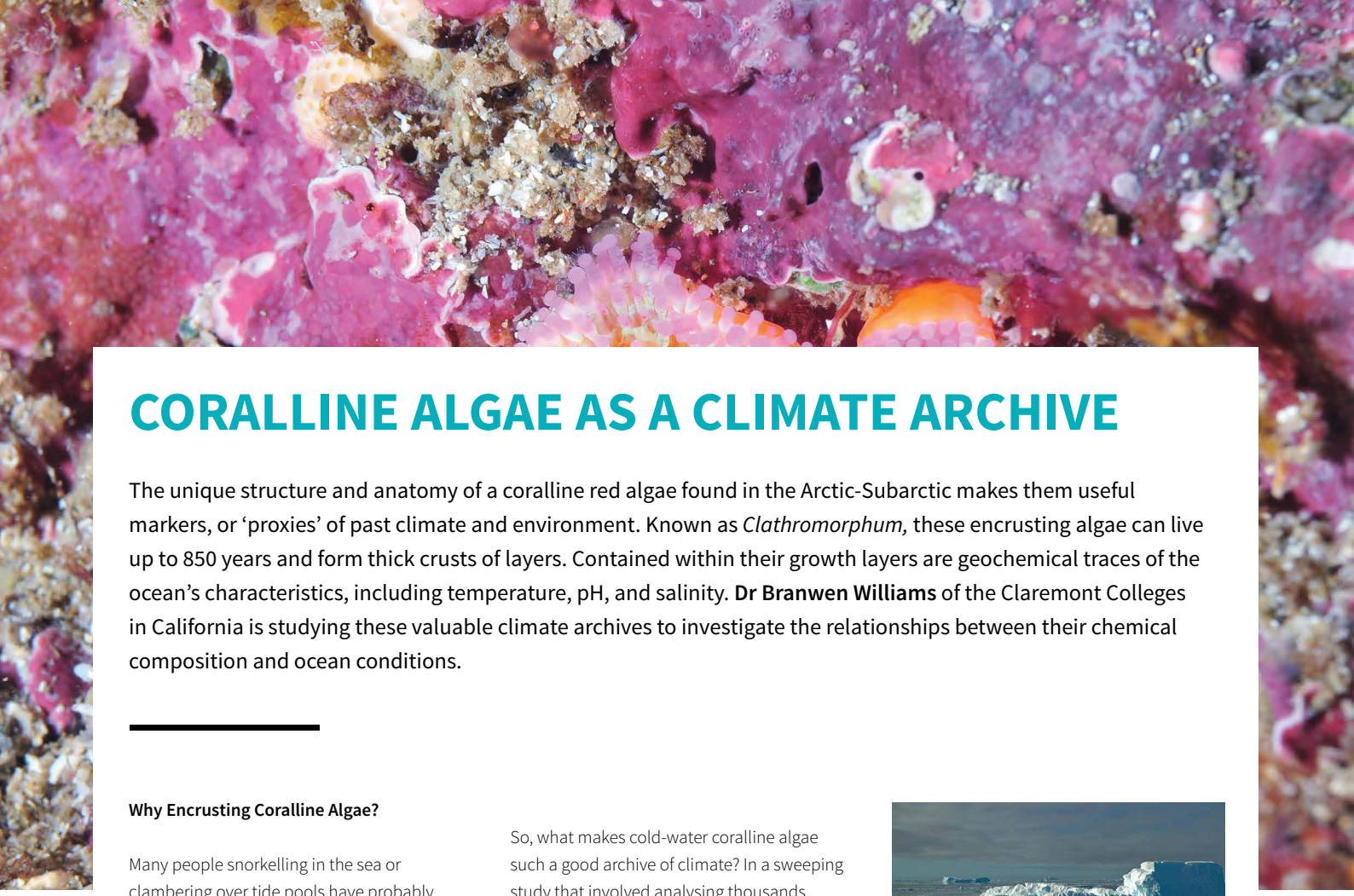
Mount Allison UNIVERSITY



CORALLINE ALGAE AS A CLIMATE ARCHIVE

The unique structure and anatomy of a coralline red algae found in the Arctic-Subarctic makes them useful markers, or 'proxies' of past climate and environment. Known as *Clathromorphum,* these encrusting algae can live up to 850 years and form thick crusts of layers. Contained within their growth layers are geochemical traces of the ocean's characteristics, including temperature, pH, and salinity. **Dr Branwen Williams** of the Claremont Colleges in California is studying these valuable climate archives to investigate the relationships between their chemical composition and ocean conditions.

Why Encrusting Coralline Algae?

Many people snorkelling in the sea or clambering over tide pools have probably seen bright pink or red blotches on rocks and corals, but have no idea what they are. These blotches are in fact a type of seaweed known as coralline red algae. Biologists also describe them as 'encrusting', since they grow by excreting calcium carbonate and slowly spread over rocks and even animals. As it turns out, these crustose algae are so widespread that they cover more area in the shallow ocean than any other algae.

Over 1,500 species of coralline algae exist, but it's the cold-water varieties that intrigue Dr Branwen Williams. Dr Williams, a geologist at the Claremont McKenna-Pitzer-Scripps Colleges in California, thinks that these algal life forms are recording climate change as we speak.

'Some marine organisms document their ambient environment in the properties of their skeleton,' Dr Williams explains. In particular, she says, coralline red algae found in high latitudes have certain anatomical features that make them better at archiving past conditions in the ocean than others. 'For example, some algae capture ambient seawater temperature in the chemical composition of their skeleton. These same algae form visible layers each year in their skeleton so that we identify when that skeleton was formed,' she says.

So, what makes cold-water coralline algae such a good archive of climate? In a sweeping study that involved analysing thousands of specimens collected along Arctic and Subarctic coastlines, scientists including Dr Williams set out to explain why.

Setting the Record Straight on *Clathromorphum*

In the past, coralline algae have gotten a bad rap. Some scientists have associated their presence with habitat that's been degraded by overfishing. The areas where they grow have even been referred to as 'coralline, sea-urchin barrens'. But Dr Williams and her colleagues dispel the notion that these algae beds are so desolate of life. They describe how dense and porous layers of coralline algae – some up to 50 cm in thickness – harbour hundreds of tiny invertebrates, much like coral reefs. They found over 700 critters such as brittle stars and chitons in one small (<1 m²) sample alone.

They defend this humble species in a 2013 report spearheaded by Dr Walter H. Adey, a scientist and curator at the Smithsonian Institution in Washington, DC. Dr Adey teamed up with both Dr Williams and another geologist, Dr Jochen Halfar of the University of Toronto, to produce the definitive report on a special genus of Arctic-Subarctic coralline algae called *Clathromorphum.*

In their 46-page report for the Smithsonian, the researchers set out to fully describe the biology, physiology and ecology of this group of algae. Their principle motivation was to highlight its significance as a chronicle of climate and environment.

One reason these encrusting algae record the paleoenvironment so well is that they live long enough to grow and develop thick layers. In their report, the team describe frequently finding 100–300-year-old specimens and others up to 850 years old in Alaska's Aleutian Sea. As they grow, the coralline algae form a natural pavement of layers called 'cor-strome', which can reach thicknesses of 50 cm.

Much like tree rings, their layers reveal both their age and clues of the past. Seawater temperature controls their growth rates and the amount of sunlight (and hence, ice cover) affects how dense they become – both reflected in their skeletons. Furthermore,

‘My work uses measurements of the skeletons of marine organisms to create records of ocean characteristics, such as a temperature or carbon chemistry. These records help us to understand how humans are changing our oceans.’

traces of seawater chemistry are permanently trapped in their growth layers.

In their study, the scientists focused on two species, *Clathromorphum compactum* and *C. nereostratum*, because of their wide distribution and unique anatomy. For one, the tough outer layer of *Clathromorphum* protects them against grazing animals, such as limpets and chitons. These invertebrates aren’t able to inflict the same damage as they can on other algae. As such, these climate archives are left relatively undisturbed and intact for centuries.

Finally, the layers of their thick skeletons contain calcite crystals. These crystals preserve the physical and geochemical nature of the ocean during their formation. For example, the 2013 Smithsonian reports that magnesium and barium are known markers, or ‘proxies’, for ocean temperature and salinity.

As it turns out, other markers found in their growth layers are chemical isotopes, a feature that greatly interests Dr Williams.

Oxygen Isotope as a Temperature Proxy

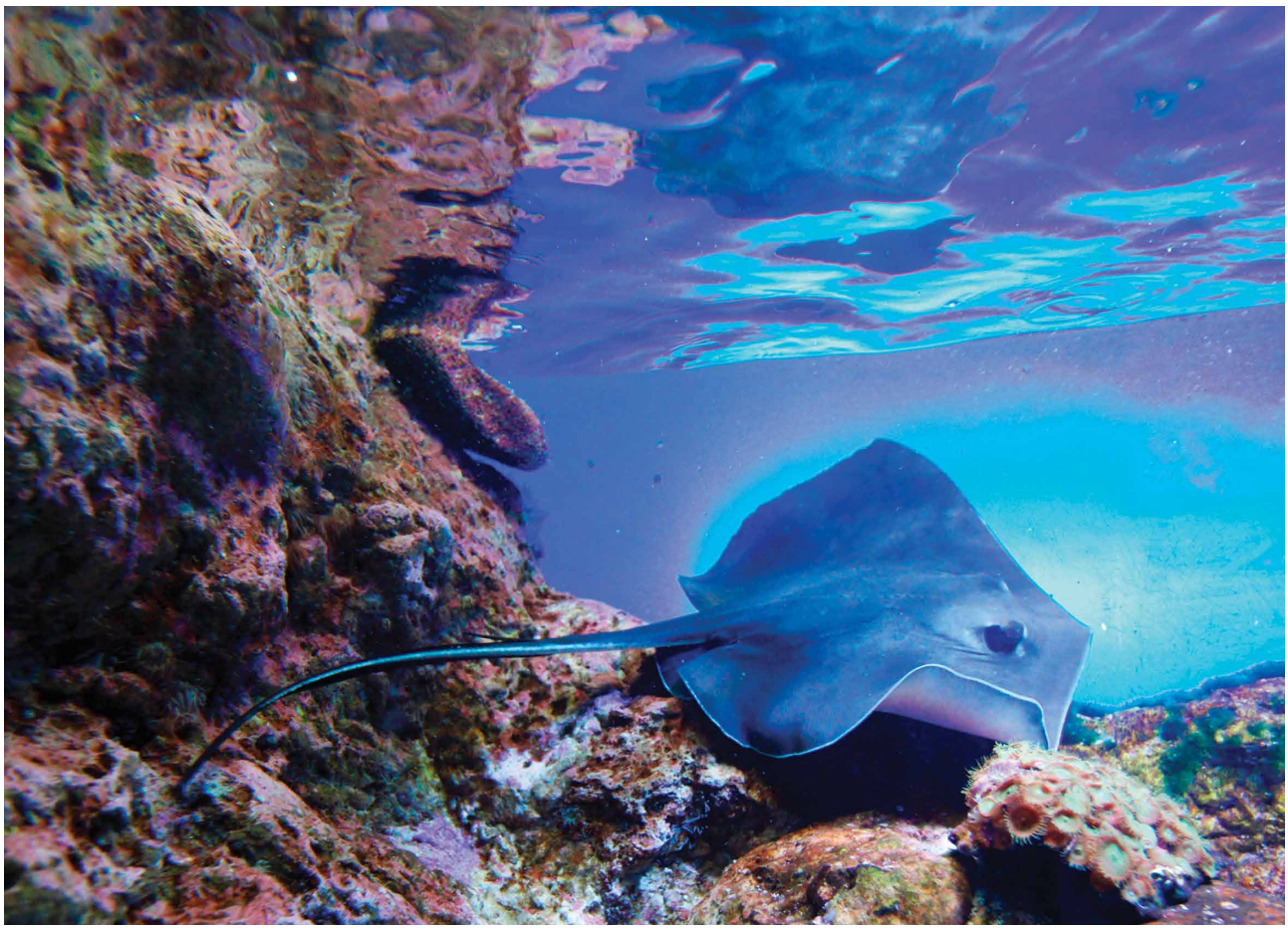
Much of Dr Williams’ research today focuses on isotopes found in coralline algae, and how they relate to historical ocean conditions. She believes that understanding the past will increase our understanding of future climate change, especially that caused by humans.

‘To learn about the human influence, termed anthropogenic, we need to have data of ocean characteristics so that we can document changes,’ she explains. ‘Such data are missing in many places, particularly in remote locations and back in time before people had thermometers or other instruments to measure the seawater characteristics.’

Recently, Dr Williams and her research group have been looking at oxygen isotopes ($\delta^{18}O$) found in *Clathromorphum* to see what clues they reveal. Similar to magnesium and barium, oxygen isotopes might reflect what seawater conditions were like during the time the algae formed its growth layer.

Dr Williams reckoned that the amount of oxygen isotopes would reflect changes in either the ocean temperature or salinity. For example, rainfall or melting ice lowers the ocean’s salinity and oxygen isotope composition. This in turn, might change concentrations of oxygen isotopes in the coralline algae.

She wanted to know whether ocean salinity or temperature was leaving a bigger mark in the chemistry of the growth layers. She and her colleagues from various institutions including Boston University and Scripps Institute of Oceanography analysed several



specimens of *Clathromorphum* ranging from the Alaskan Aleutian Islands to the Gulf of Maine. After determining their ages, they compared their $\delta^{18}O$ concentrations to known ocean temperatures and salinity from the same time. They found that oxygen isotopes more closely matched changes in ocean temperature.

The researchers presented their findings at the fall American Geological Union meeting. They found that oxygen isotopes found in coralline algae may be a good climate archive for sea surface temperatures. The next step in developing the proxy system model is to test if algal growths also impact oxygen composition.

Do Boron Isotopes Record Ocean Acidification?

Now, Dr Williams and her team are chasing boron isotopes in their search for another climate archive, in this case, ocean acidification.

Since the beginning of the Anthropocene – our current geological age that started with the Industrial Revolution in the early 1800s – humans have emitted tons of carbon dioxide into the atmosphere. Oceans have mitigated in some way by absorbing around one third of this carbon dioxide, but not without consequences on their overall chemistry and health.

When seawater absorbs carbon dioxide, carbonic acid is formed. This chemical reaction lowers the pH. The formation of carbonic acid also depletes carbonate ions, which directly affects marine life. Animals such as shrimp and corals need carbonate ions to grow shells, just as coralline algae rely on them to accrete calcite and form crusts.

This process, known as biological calcification, slows down without carbonate ions. Colder water absorbs carbon dioxide more readily than warmer water, making Dr William's study sites in the Arctic more vulnerable to ocean acidification.

Since the Anthropocene begun, the pH of the global oceans has decreased from 8.2 to 8.1, which will undoubtedly affect marine life. Dr Williams thinks that coralline algae might be a 'first responder,' or one of the first to suffer from increased acidity. However, she says little is known about changing seawater pH in their habitats.

For that reason, Dr Williams and her team are looking at how declining seawater pH in the Subarctic-Arctic might affect the growth of coralline algae. They are specifically homing in on boron isotopes ($\delta^{11}B$). As it turns out, the ratio of boron isotopes found in the calcite skeletons of coralline algae appear to decrease with decreasing seawater pH. Because these organisms are so long-lived, the ratio of boron isotope detected in their growth layers might make an ideal proxy for seawater pH.

Finally, the nature and anatomy of coralline algae itself – in particular the *Clathromorphum* genus – might make it the ultimate climate archive. This is widely-distributed, long-lived and has well-defined annual growth layers.

'We can create records of past environmental change by measuring the changes in their chemical composition,' says Dr Williams says of her valued subject. 'With these records, we can then start to see how our oceans change, both naturally and as a result of human activities. We can thus infer the "health" of our oceans.'

Meet the researcher

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Dr Branwen Williams is an Assistant Professor at the Claremont Colleges in California, where she teaches courses in oceanography, environmental science and climate change. She received her PhD in 2009 in Geological Sciences from Ohio State University after obtaining her MSc in Biology at the University of Quebec at Montreal in 2005. She has received funding and awards from key scientific organisations including the National Science Foundation, National Geographic and the National Oceanic and Atmospheric Administration. In 2013, Williams co-authored 'The Coralline Genus *Clathromorphum* Foslie emend. Adey: Biological, Physiological and Ecological Factors Controlling Carbonate Production in an Arctic-Subarctic Climate Archive' for the Smithsonian Institution. Recently featured in National Geographic's Voices program, Dr Williams travels to remote places such as Nunavut, Canada and the Kiribati islands in the Pacific for her research. She has also been invited to serve as guest lecturer and panellist for several workshops relating to her studies of coralline algae and climate change.

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INVESTIGATING METALS IN THE WORLD'S INVERTEBRATE ANIMALS

Ecotoxicologist Professor **Reinhard Dallinger** and his colleagues at the University of Innsbruck in Austria look for ways to locate and measure environmental metal fluxes and pollution in non-model invertebrate and indicator organisms, like worms and shellfish.

Looking for Metals that Pollute Our World

Today when one thinks about metals, perhaps it's in the context of the perilous economic environment the world has been 'enjoying' lately. Converting pounds, dollars or euros into gold, silver or other precious metals may be an investment strategy that makes sense – metals, not potentially worthless paper. Of course, medical and health conscious individuals more likely think of metals such as iron to prevent anaemia or calcium to strengthen bones. Those metals – along with zinc, copper, manganese and others – are necessary for proper tissue metabolism and health. But environmentally conscious folks are more likely to think of hazardous metals, such as lead, mercury or cadmium – metals resulting from human industry that contaminate our world and even cause human disease. The reason why this matters resides in the fact that normally solid metals as encountered in many metallic products are converted into ionic metal ions by atmospheric oxidation, thus becoming easily available to biota in the environment including marine, freshwater and terrestrial ecosystems. This is where Professor Reinhard Dallinger and his colleagues at the University of Innsbruck concentrate their efforts – utilising fundamental research techniques to characterise metal metabolism, regulation and detoxification in various organisms with ecological implications and combining that with applied research to make advances in various areas of ecotoxicology.

Metals, Metals Everywhere – Good, Bad and Ugly

One feature of metals is that they generally conduct electricity. Each time we turn on the lights, we're using a metal – commonly copper – to conduct the electricity to the light bulb. But we also use metals unconsciously each time we take a breath of air. The red blood cells that carry oxygen from our lungs to our tissues use iron atoms present in haemoglobin molecules to carry oxygen throughout the body. Without enough iron, our blood cannot carry enough oxygen to sustain proper cellular functioning. Thus, although present in small amounts, these so-called essential metals are extremely important in bodily metabolic functions, both in humans and in other animals. They can become toxic, however, if their concentrations in biota exceed tolerable levels.

But just as there are beneficial, even vital, metals that participate in our biology, there are also metals in our environment that can be dangerous at even low concentrations, sometimes extremely so. They are called non-essential metals. One well-known example is the outbreak of so-called Minamata disease in Japan in the 1950s. Ultimately, over 2,000 people were affected with severe mercury poisoning due to industrial dumping of methyl-mercury compounds into Minamata Bay and the Shiranui Sea near the city of Minamata in Japan. Deaths, foetal malformations and severe neurological disease resulted from mercury poisoning before the contamination was identified and halted. Although mercury exists in nature in small amounts, exposure and absorption of large amounts cause severe effects. Even now, after widespread regulation against mercury contamination of the environment, pregnant women are advised against eating large ocean-going fish like shark, swordfish, king mackerel, or tilefish, due to higher than usual levels of mercury that may cause foetal malformations.

Another metal contaminant that can cause significant environmental problems, as well as human disease, is cadmium. Although

‘I try to connect questions of fundamental ecotoxicology with the fields of molecular physiology, biochemistry, evolutionary biology and ecology. In fact, I love to work at the interface between different disciplines and across different levels of biological organization – from molecular through cellular, organismic and ecosystemic.’

cadmium exists in the Earth’s crust in small amounts, on the order of between 0.1 and 0.5 parts per million, higher concentrations result from the mining of zinc ore. The use of cadmium in such processes as metal plating, plastic production and nickel-cadmium batteries can result in contamination of the environment with unnaturally high levels of cadmium. This can lead to human disease, including cancer. In fact, cadmium has been declared a human carcinogen by the International Agency for Research on Cancer, the US Environmental Protection Agency (EPA) and the European Chemical Agency. As far as we know, cadmium has no biological function, at least in higher organisms. However, since cadmium does exist in nature, it is logical that organisms have mechanisms to allow them to excrete cadmium that they happen to ingest or absorb. This mechanism is what Professor Dallinger and his colleagues study to find out how different organisms adapt and respond to exposure to otherwise toxic metals, and what the implications of these response mechanisms are from a physiological, biochemical and ecotoxicological perspective.

Working Across Disciplines and Levels of Organisation

Professor Dallinger tells Scientia that since his early days at university, he has been interested in a synthesis approach to his work, rather than a specialist approach. He has studied zoology, microbiology, geology and mineralogy, and he puts all of that knowledge into his work in ecotoxicology. He attempts to connect questions of fundamental ecotoxicology, particularly the understanding of mechanisms of ecotoxicity, with the fields of molecular physiology, biochemistry, evolutionary biology and ecology. He is driven to work at the interface between different disciplines – what he calls *interdisciplinarity* – and across different levels of biological organisation, from the molecular level through the cellular level to the organism and then the entire ecosystem. Although this may seem very ambitious, some of his published work in different fields may have begun to achieve this goal. At the same time, this kind of research offers Professor Dallinger the opportunity to connect fundamental science with actual real-life applications.

As examples of this interdisciplinarity and cross-organisation approach in action, three of Professor Dallinger’s recent grants, funded by the Austrian Science Foundation, addressed certain metal-binding molecules – metallothioneins – in species of snails. In one study, Professor Dallinger and his colleagues looked at the *molecular* basis for stress response plasticity in cadmium-metallothionein genes of snails. In another study, they investigate

cell-specific interactions of snail metallothionein. In yet another study, the team take a multi-disciplinary approach to the *evolution* of snail metallothioneins towards structural and functional metal selectivity. From molecular to cellular to evolutionary and across disciplines – this is how Professor Dallinger likes to attack his research topics.

Looking at the Big Picture

While Professor Dallinger's work seems to focus on molecular functions – metals and molecules – he is really interested in something more global. Basically, he wants to know how animals can adapt to specific kinds of stressors in their environment – in this case, metals like cadmium or copper – by focusing on the mechanisms and evolutionary processes that make adaptation possible. He follows the function of specific enzyme and molecular systems to see how animals solve the problem of contamination by possibly toxic agents like cadmium. At the same time, he's interested in the modulation and optimisation of these mechanisms through evolution on different time scales, including the phenomena of micro- and macroevolution. A good example may be the question about how metallothioneins may have evolved through different animal lineages from rather unspecific metal binding molecules towards highly metal-selective proteins.

In one paper published in the journal *BMC Biology,* Professor Dallinger and his group looked at a specific mollusc species, the Roman snail, and its metallothionein molecules to understand how metallothioneins may have developed evolutionarily. They found two different isoforms of metallothioneins in the snail – one specific for cadmium and one specific for copper. Clearly, the snail had evolved to process the two metals differently, since copper is a beneficial metal used in various metabolic processes, while cadmium is toxic and must be excreted. After comparing these results with known metallothionein forms from other species, the team concluded that these metal-specific metallothionein forms might be regarded as prototypes of metallothionein families that evolved genuine metal-specificity within the animal kingdom. Diversification into these metallothionein forms may have been caused by gene duplication perhaps, followed by speciation and selection towards different needs for protecting copper-dominated metabolic pathways from nonessential (and possibly toxic) cadmium. The mechanisms enabling these metallothioneins to be metal-specific could also be relevant for other metalloproteins from other species besides just molluscs.

Small Animals, Big Environmental Effect

Another focus of Professor Dallinger's work has been studying the biochemical and cell-physiological mechanisms that govern the accumulation of metallic trace elements in various invertebrates and fish. By looking at the physiological and ecological aspects of metals as they travel through the ecosystem and are absorbed and excreted by these animals, he has expanded our knowledge and understanding of the importance of various invertebrate species for the trace element transfer in ecological habitats. He has also looked at the use of some representative species as biological indicators of metal contamination in the areas they inhabit. In one study published in the journal *Oecologia*, Professor Dallinger and his colleagues looked at lead and cadmium levels in a species of woodlouse around the environs of Innsbruck and detailed the distribution of the metals around the city. This gave them a picture of the directional spread of the metals from industrial sites where contamination occurred.

Professor Dallinger's team has also focused on aspects of microevolution and adaptation of populations to contaminants, such as metals and pesticides, in combination with different man-made stressors. They found, for example, that a widespread species of moth that attacks apple trees split into different adaptable populations over small-scale areas, apparently due to selective pressure from the application of pesticides. Some of the populations actually acquired pesticide resistance. Another example of microevolutionary adaptation to environmental pollution was discovered in populations of the freshwater tubifex worm. Prior and recent metal pollution in some European river systems have caused tubifex populations to split into groups that differ with respect to their resistance to metal contamination. So, whether it's worms, moths or molluscs, Professor Dallinger will follow the ecotoxicology trail wherever it leads and at whatever level necessary to expand our knowledge and understanding.

What's Up for the Future?

Professor Dallinger tells Scientia that he ultimately wants to develop a solid overview on the history of evolutionary optimisation of stress response in a number of animal phyla. He is doing that by following the development of the metallothionein system in different species and getting down to the molecular level. In a paper, he and his colleagues recently submitted for publication, they report a nuclear magnetic resonance analysis of metallothionein forms from a species of littoral snail. They found that this snail's metallothionein was constructed in three individual domains, each folding into a single well-defined three-metal cluster. This allowed the molecule to bind to either nine zinc or cadmium ions, three in each domain. They suspect that the expression of a three-domain metallothionein confers to the snail an evolutionary advantage in coping with cadmium contamination and adverse environmental conditions.

This study illustrates Professor Dallinger's dream – multidisciplinary experts from centres across Europe performing complex chemical analysis on molecular structures to address a question of evolutionary design. This is just what he has always wanted – his interdisciplinarity addressing the multi-levelled problems to obtain the big picture.

Meet the researcher

Professor Reinhard Dallinger
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Professor Reinhard Dallinger received his PhD from the Faculty of Natural Sciences at the University of Innsbruck in 1978, for a thesis involving ecophysiology research focusing on the copper metabolism of terrestrial isopods. After seeking further training in archaeology, aquaculture, ethnology and environmental physiology – including an expedition to Lake Tanganyika in Africa – he joined the faculty of the University of Innsbruck in 1997, where he is currently Professor of Ecotoxicology there. Professor Dallinger's main research interests are directed towards studying the adaptation, response patterns and molecular reactions of animals towards stress factors, primarily factors caused by human effects on the environment. In particular, he focuses his attention on the study of invertebrates and their importance for trace element transfer in terrestrial and aquatic habitats and for the application of some key representative species as biological indicators in metal-contaminated environments. He feels that in this area there is a particular need to integrate fundamental and applied environmental research methods.

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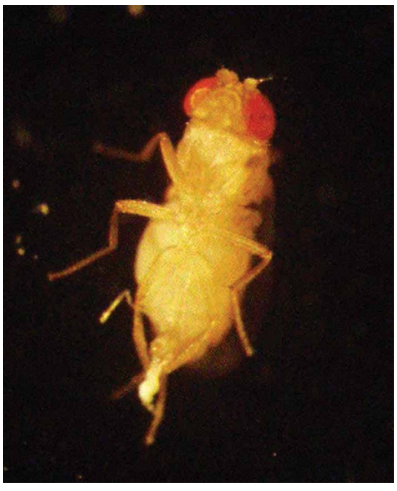
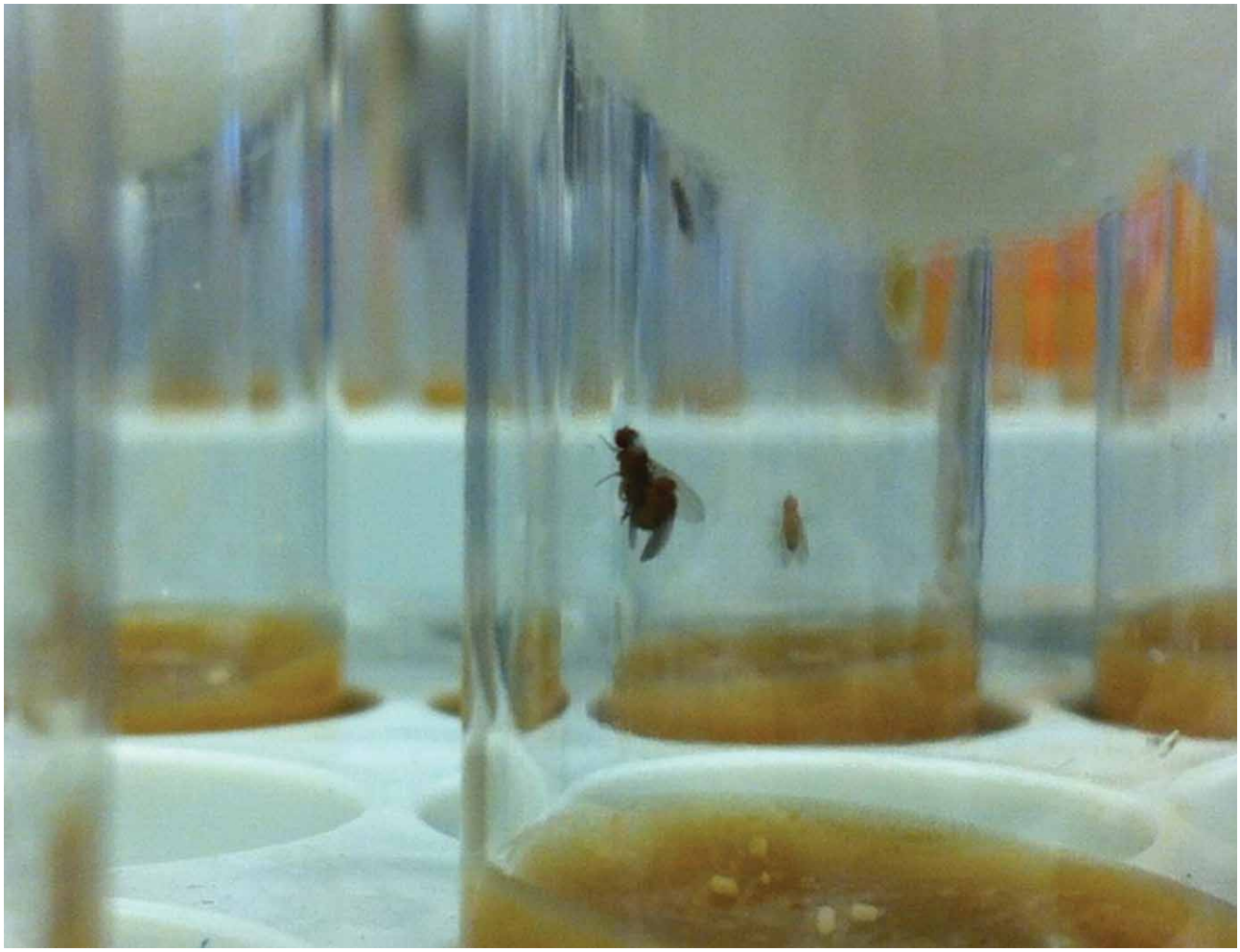
HOW FEMALE FLIES CHOOSE THE RIGHT PARTNER AFTER MATING WITH MULTIPLE MALES

Research led by **Dr Mollie Manier** at The George Washington University has revealed how female fruit flies choose the right sperm to fertilise their eggs after mating with multiple males.

Secondary sexual male ornaments are advantageous in acquiring mates and can be found throughout nature, from the antlers of a stag to the mandibles of a stag beetle. The peacock's iconic tail feathers confer higher reproductive success for males with the longest and brightest tails, because they are preferred by female peahens, despite hindering escape from predators. Thus, sexual selection is a powerful force driving the evolution of traits that influence reproductive success, even at a potential cost to the individual carrying them. We are most familiar with secondary sexual traits that influence an individual's mating success, but sexual selection can continue after mating in the form of 'postcopulatory sexual selection', which can drive rapid diversification of equally impressive male primary reproductive structures directly involved in mating (i.e., sperm or genitalia). The most extreme example is found in the lowly *Drosophila* fruit fly, a classic model organism for studying many aspects of biology, including molecular and evolutionary genetics. These small flies boast among the longest documented sperm cells, varying from 0.32 mm to an incredible 5.8 cm – 20 times the length of the fly's body and around 1000 times longer than human sperm cells.



'In the fruit fly *Drosophila*, we have documented three separate mechanisms by which females can influence paternity – timing of sperm ejection from the female reproductive tract, use of different sperm storage organs, and the length of the seminal receptacle'



Female fruit flies retain sperm from multiple males within specialised sperm-storage organs in the female reproductive tract, where they engage in sperm competition for fertilisations, a phenomenon that is widespread in many species. Historically, scientists assumed that the female reproductive tract serves only as an arena in which sperm compete for access to the egg and disregarded the potential for female mediation of paternity success through 'cryptic female choice'. Although cultural biases contributed to this trend, investigating interactions within the female reproductive tract is fundamentally more difficult than simply focussing on sperm alone. However, Dr Mollie Manier and her team at The George Washington University have shown that the fate of sperm from different males may be determined by morphological, behavioural, or physiological characteristics of the female. Thus, the female fly can exhibit 'preference' for different mating partners long after the actual matings have occurred. Female flies can influence which sperm fertilises their eggs in several distinct ways, the primary method being the timing of sperm ejection.

Sperm Ejection from the Female Reproductive Tract – It's All About Timing

During mating, male fruit flies transfer many more sperm into the female's 'uterus' than can be stored. This pool supplies a constant stream of sperm entering the sperm storage organs, where they physically displace sperm from her previous mate. Female ejection of excess sperm from the uterus terminates this displacement process, and the proportion of sperm from different males remaining in storage determines their paternity success. As a general rule, longer ejection times favour the second male by allowing his sperm more time to enter storage and displace rival sperm. Dr Manier and her team have recently shown that ejection time by female fruit flies depends on the attractiveness of her mate. Females ejected the second male's sperm sooner if the first male was preferred and later if the second male was the best catch. This ejection timing is thought to be a primary mechanism of cryptic female choice, because it is consistently present in all species examined thus far, though not all species necessarily use it to bias fertilisation to favour certain males.

The timing of ejection is likely to play a role in cryptic female choice if ejection occurs before displacement equilibrium is reached.

Displacement equilibrium occurs when the proportion of sperm in storage from, say, the second male reaches the same value as that in the uterus. At this point, sperm entering storage from the bursa is equivalent to sperm already present in storage, and thus no further displacement can occur. If ejection occurs before this equilibrium is reached, females can influence the proportion of the second male's sperm in storage and thus paternity success. The research team found that female preferences for males is based on traits such as body size and whether the male is a member of the female's species. These characteristics act at multiple stages in reproduction, such that females mate more rapidly with more attractive males and also eject their sperm later, presumably after displacement equilibrium is reached. The lab also discovered that females use the first male they mate with as the basis for evaluating subsequent mates. The ejection time for a preferred male is only longer if the female mates with a less preferred male first. Likewise, ejection time is only shorter if an unattractive mate follows an attractive male in the mating order.

How Different Sperm Storage Organs Influence Paternity

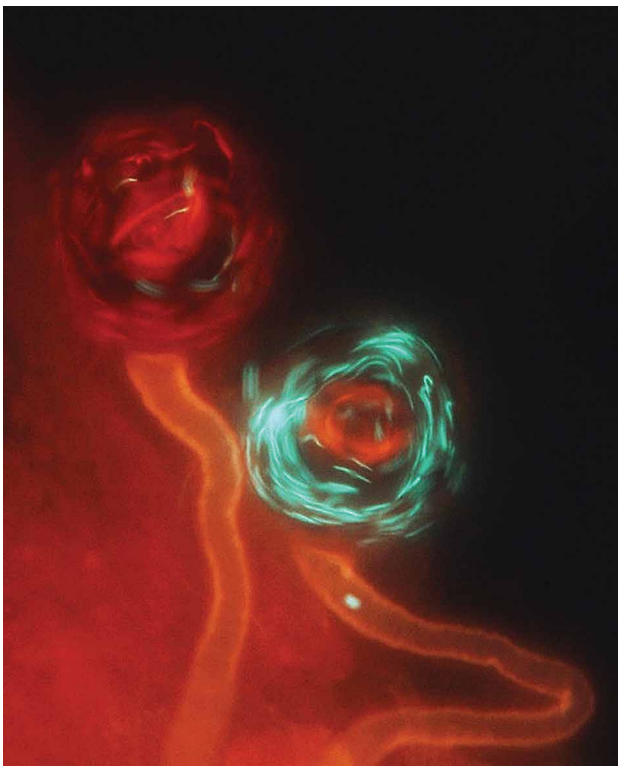
The increased complexity of the female reproductive tract is thought to give females more opportunities to control which male fertilises her eggs, because fertilisation bias can then come from multiple sources. Fertilisation bias occurs when sperm use deviates from an expectation of 'fair raffle', that is, sperm use that reflects the proportion of sperm stored from different males. In other words, if one male's sperm are used disproportionately, above and beyond their numerical representation in the sperm storage organs, fertilisation bias can be invoked.

As a case in point, female *Drosophila* store sperm within two distinct types of sperm storage organ that differ in structure and function, and Dr Manier found that both can display different patterns of fertilisation bias. In *D melanogaster,* the long, coiled seminal receptacle is the primary sperm storage organ from which sperm are used first for fertilisation, while the pair of mushroom-shaped spermathecae seem to be for long-term sperm storage. The spermathecae also secrete proteins and metabolites that are critical for sperm storage in both the spermathecae and seminal receptacle. The process of displacement of rival sperm by a second male's sperm described above only occurs in the seminal receptacle. Within the spermathecae, the second male's sperm simply 'tops off' the female's sperm stores without any measurable displacement.

Dr Manier's team found that closely related species show different patterns of fertilisation bias. While *D melanogaster* favours using the seminal receptacle over the spermathecae, *D mauritiana* appears to show no fertilisation bias. At the same time, *D simulans* females exhibit first-male bias in sperm from their seminal receptacle but second male bias from their spermathecae. Moreover, they alter which storage organ is used for fertilisation depending on whether the preferred male was first or second in the mating order.

Coevolution of Sperm and its Receptacle

The third mechanism of cryptic female choice is mediated by the length of the seminal receptacle, which appears to drive the evolution of giant sperm in *Drosophila.* In *D melanogaster,* long sperm outcompete short sperm but primarily in long seminal receptacles. This 'long sperm advantage' seems to be due to a superior ability for longer sperm to



displace (when mating second) and resist displacement by shorter rival sperm (when mating first), likely arising from mechanical fluid dynamics generated by longer flagella within the seminal receptacle.

Selection for longer sperm by longer seminal receptacles may explain why species with longer sperm in males also have longer seminal receptacles in females, while species with shorter sperm have shorter seminal receptacles. Although it is difficult to completely isolate the role of the female (played out through female reproductive tract morphology or physiology) from that of the male (via sperm or seminal fluid attributes), sexual selection theory predicts a coevolutionary dynamic between males and females over the control of fertilisation. In *Drosophila,* this coevolutionary process is driven by a genetic link between the genes that control sperm length and seminal receptacle length. In other words, genes controlling both of these traits are expected to be clustered within the fly genome. Some genes might even be important in both spermatogenesis and development of the seminal receptacle during fly metamorphosis.

Another aim of Dr Manier's research is to identify genes involved in sperm length and seminal receptacle length, investigate how they might have coevolved at the molecular level, and determine if any genes are shared by both traits. On the wider impact of her work, Dr Manier explains that the implications of this research extend beyond increasing our understanding of how fruit flies reproduce. The team is shedding light on the genetic and molecular mechanics behind coevolution, which is useful in better understanding HIV, cancer, and human fertility. Many couples who are unable to conceive are thought to be 'genetically incompatible', for reasons unknown. A better understanding of what might cause this incompatibility could potentially lead to new treatments for infertility.

Meet the researcher

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Dr Mollie Manier studied for her undergraduate degree at UC Berkeley where she became fascinated with natural history and population genetics while working in the Museum of Vertebrate Zoology. She then went on to pursue a PhD degree at Oregon State University, where she studied the metacommunity landscape genetics of two garter snake species and a toad. She has also worked at Hopkins Marine Station, in Monterey Bay on the evolution of sea urchin sperm and later at Syracuse University, where she began researching the fruit fly *Drosophila.* She now runs her own research group at the George Washington University, in Washington DC.

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Two Utah prairie dogs kissing early in the morning while scanning for predators. CREDIT: Elaine Miller Bond (www.elainemillerbond.com)

PRAIRIE DOGS ARE CHARMING AND CUTE, BUT THEY ARE ALSO SERIAL KILLERS

When **John Hoogland** first visited a prairie dog colony as a young graduate student, he said aloud to himself, 'I could study these animals for the next 10 years.' Forty-four years later, John is still studying prairie dogs, and he still marvels about the sometimes bizarre, often provocative, and always fascinating lives of his favourite animals. He is the undisputed world's expert on these remarkable social rodents.

CREDIT: Mariana Rivera Rodriguez

John Hoogland reading the eartags of one of his Gunnison's prairie dogs; note the canvas bag used for handling.

Behavioural ecologist Professor John Hoogland's research career began with a deceptively simple question: Why do animals live in colonies? To pursue the answer, he turned to prairie dogs, which are diurnal, herbivorous, squirrel-like rodents that live in large colonies throughout the grasslands of western North America. These gregarious rodents, whom John frequently calls his 'little people', have afforded him unique opportunities for his research over the last four decades, with sweeping conclusions for not only prairie dogs, but also other social animals.

Life in the Colony

In April 1974, while working on a PhD at the University of Michigan, John found himself in a devastating situation for a young graduate student. While attempting to study colony dynamics of Wyoming ground squirrels, he discovered that, contrary to popular belief at the time, Wyoming ground squirrels do not actually live in discrete colonies. One night John broke down and sobbed to his new bride (Judy) about his calamity. Judy instantly responded, 'Why not study prairie dogs? Everybody knows that prairie dogs live in colonies.' John listened, and visited a colony of prairie dogs the next day. Within a few minutes of his arrival, John was mesmerised by the nonstop vocalisations, and by the chases, fights, and kisses that he was seeing everywhere.

John quickly developed the simple protocol of 'Catch 'em. Mark 'em. Watch 'em.' He and his students – usually 4–5 per year, with a grand total over 200 – took this protocol to a new level over the next 44 years: an inconceivable >200,000 man-hours of research with four different species of prairie dogs under natural conditions at national parks in Arizona, Colorado, New Mexico, South Dakota, and Utah. And yet John still says at every one of his lectures, 'The more I learn about prairie dogs, the less I know.'

John's initial research focused on trying to figure out why prairie dogs live in colonies. When compared with solitary prairie dogs, colony residents face higher competition for food, mates, and other resources, and they also incur a higher risk for contracting nasty diseases and parasites.

But what are the compensating advantages that over-ride these serious costs? John's early research showed that the major benefit to living in a colony for prairie dogs is the lower risk of being captured by a predator. 'With so many individuals within a colony watching for enemies, the probability that a bobcat, coyote, or golden eagle will be able to capture a prairie dog is low,' John says.

‘Long term research and careful analyses are crucial and decisive for understanding the ecology and social behaviour of animals as complex as prairie dogs. I have been fortunate to spend a lifetime studying these captivating keystone species. Consequently, I have been able to make several riveting discoveries that have led to novel, far-reaching conclusions.’

CREDIT: Elaine Miller Bond (www.elainemillerbond.com)
Utah prairie dog male attacking another male during the mating season.

The alarm call in response to predators is a pivotal reason for this increased safety. But alarm calling is puzzling. Why should an individual draw a predator’s attention to itself by calling in order to save other prairie dogs with whom it competes? The payoff is that alarm calls warn genetic relatives of danger. These calls warn nearby offspring for three of the prairie dog species that John has studied. For the fourth species, individuals call not only for offspring, but also for more distant kin such as nieces, nephews, and cousins.

Predation of prairie dogs is rare, as just noted. When it occurs, John was expecting that the old, the weak, and the sickly would be the most common victims. But the prairie dogs surprised him. He learned that the largest, most aggressive, and most successful males in one species are the most susceptible to predation during the mating season – because they are so obsessed with finding and inseminating receptive females, with little fear of predators. And for the 3rd and 4th weeks after they mate, females in late pregnancy are especially vulnerable – because they are overweight with as many as 8 large foetuses, and therefore unable to run as fast as other prairie dogs.

Family Matters

Family dynamics can be complicated when your entire extended family lives in a small area, as occurs for all four species of prairie dogs that John has studied. One of his early objectives after graduate school was to figure out if prairie dogs are concerned about incest. The solution did not come easily, because the mating season lasts for only 3 weeks in late winter and early spring at high altitudes, where temperatures are low and arctic winds are relentless. Further, each female is sexually receptive for only several hours on a single day. And finally, most copulations occur underground! By watching marked individuals from dawn to dusk every day, by watching for diagnostic aboveground behaviours just before and just after an underground copulation, and by collecting DNA-samples from all juveniles and all potential parents, John and his students are able to specify who mates with whom for over 95% of females at their study-colony each year. By following parents and their offspring – for a minimum of 10 years for each of four species – John has documented that prairie dogs consistently avoid incestuous matings with close kin such as parents, offspring, and siblings, even though these genetic relatives frequently live in the same or adjacent territories. Such avoidance reduces the problem of inbreeding depression (the reduction of survivorship of inbred offspring). But prairie dogs frequently mate with more distant kin such as aunts

Two Utah prairie dogs on the first day they appear aboveground at weaning, when they are about 5.5 weeks old and weigh about 200 grams.

and uncles, nieces and nephews, and first and second cousins. So they avoid extreme inbreeding (incest), but prairie dogs regularly engage in moderate inbreeding.

John's careful observations of the prairie dog mating system have provided insights for another conundrum first recognised by Charles Darwin almost 150 years ago: polyandry (i.e. mating with more than one male). 'Females of almost every sexual species that has been carefully studied – from plants and slime moulds through humans and other primates – commonly solicit sperm from two or more males,' he explains. 'But this is a paradox, because a female can almost always obtain all the sperm she needs to fertilise all her eggs from a single mating, and additional matings increase the risk of diseases, parasites, and predation.' John has documented three clear benefits for female prairie dogs that mate with more than one male: polyandrous females are more likely to conceive, they wean larger litters, and their offspring have higher survivorship in the first year after weaning. Win, Win, and Win again. For prairie dogs, at least, polyandry is no longer a mystery – with the implication that these same benefits might apply to the thousands of other less-studied species for which females frequently mate with more than one male.

Beyond the dynamics of choosing mates, prairie dog mothers face other formidable challenges associated with raising offspring in a crowded colony. Both invading adult males and lactating females commonly kill and cannibalise a mother's unweaned offspring, for example, and this infanticide is the main cause of juvenile mortality in most years. Lactating black-tailed prairie dog females are especially likely to kill, and murdered most or all of the juveniles for a whopping 65 of 294 litters (22%) under surveillance by John and his students over several years. These 65 mass killings involved 38 different females, and, incredibly, the victims were the offspring of close kin in the killer's home territory in 62 of the 65 killings (95%). The most common victims were the offspring of the killer's mother (the killer's siblings), the offspring of the killer's daughter (the killer's grandoffspring), and the offspring of the killer's sister (the killer's nieces and nephews). Some lactating females were more likely to kill than others. One serial killer, for example, slew all juveniles in eight litters over 3 years, and another serial killer dispatched juveniles in six litters over 3 years. But now consider this: When juveniles are about 6 weeks old and appear aboveground from their nursery burrows for the first time, females commonly suckle the offspring of close kin such as sisters, daughters, and nieces living in the home territory – the same offspring they had been trying to kill for the previous several weeks. Say what?!? 'Life for prairie dogs is all about balancing competition with cooperation,' says John, 'but this sudden transition from the killing of the offspring of close kin to the communal nursing of the offspring of close kin is nonetheless extraordinary.

Though this balance of competition versus cooperation can be difficult to maintain, John has found that females usually remain in the natal territory with other family for their entire lives. But why do a few females disperse? John ignored this question for over 35 years, until Judy wanted to know more about dispersing females. 'It's so rare, it's not worth further investigation,' John replied to his wife. But Judy would not let it go. When John agreed, begrudgingly, to do an analysis for one species, he immediately realised that Judy's persistent curiosity had led him to a stunning discovery: Females only disperse when they have no surviving close kin in the home territory. When John did the same analysis for the other three prairie dog species, he found the same unexpected pattern. Indeed, females are 12.5 times more likely to disperse in the absence of mother and siblings for one species, and 5.5 times more likely for another species. This striking pattern of dispersal has previously been detected in only one other species – even though dispersal is one of the most intensively studied topics in all of ecology. For female prairie dogs, the benefits of cooperating with family members (e.g. alarm calling, communal nursing) evidently outweigh the costs of competing with the same family members (e.g. fighting, infanticide). So females stay home and rarely disperse. And for John, the benefits of listening to his wife are enormous, with no costs.

The Latest Outlandish Discovery

Individuals of different species commonly compete with each other. African lions and spotted hyenas compete for the same prey, for example. To reduce interspecific competition, natural selection commonly favors divergence in ecology, morphology, or physiology. But natural selection has favored a different, unusual strategy for one species of prairie dog that John has studied: White-tailed prairie dogs kill, but do not consume, Wyoming ground squirrels encountered in the prairie dog's home territory. Like prairie dogs but smaller, ground squirrels are colonial, diurnal, burrowing rodents that inhabit western North America and eat the same plants that prairie dogs also consume.

In collaboration with Charles Brown, John discovered that interspecific killing (IK) of ground squirrels by prairie dogs was common, involving a preposterous 47 different killers of both sexes. In 3 of 6 years, the number of IKs was higher that the number of ground squirrels killed by the combined efforts of fifteen mammalian and avian predators such as American badgers,

coyotes, and Swainson's hawks. In a typical IK, the prairie dog repeatedly bites the ground squirrel in the neck or thorax over a period of 1–3 minutes until death, and then walks away with no consumption. Most killers (N = 28) slew a single ground squirrel, but 19 serial killers slew two or more ground squirrels. One female killed six ground squirrels over 5 years, another female killed nine over 4 years, and a third female killed seven juveniles from the same litter in a single day. John's large sample size (N = 163 IKs, over 6 years) allowed him to demonstrate that females that kill ground squirrels have significantly higher annual and lifetime reproductive success than nonkillers. These differences in Darwinian fitness probably result from lower interspecific competition with ground squirrels for vegetation following IKs – so that killers and their offspring consequently have more food.

Because IKs were so quick, subtle, and unanticipated, John and his students studied white-tailed prairie dogs for 4 years before they detected the first case. Their compelling results should help other ecologists to realise that IKs might be happening frequently but surreptitiously, and with significant consequences for Darwinian fitness, for animals they have been studying for many years.

The Future of the Prairie Dog

Prairie dogs have faced many serious challenges over time, mostly from humans. Because prairie dogs are herbivores that commonly live in grasslands used for raising cattle, ranchers have worried that they will consume vegetation necessary to fatten their livestock. Such competition does sometimes occur, but cattle nevertheless often prefer to forage at colony-sites because the plants there can be more nutritious. Ranchers also fret that their cattle will incur broken legs from stepping into burrows, but such fractures are exceedingly rare. These overrated concerns have led ranchers to shoot and poison billions of prairie dogs over the last 150 years, often with assistance from federal and state agencies. In recent years, disease has been another problem for prairie dogs: Millions have died from epidemics of bubonic plague, an introduced disease to which prairie dogs have no good defence. Further, housing communities and shopping malls have destroyed much of their natural habitat. As a result, two species of prairie dogs are now on the list of threatened and endangered species, and the other species probably should be on that list as well.

John Hoogland releasing a Utah prairie dog uniquely marked with three vertical stripes for identification from as far away as 100 m.

John has documented that prairie dogs have a profound impact on their grassland ecosystems, and are therefore keystone species. For example, their burrows change the landscape and soil chemistry, affect the cycling of water and nutrients, alter floral species composition, and provide shelter and nesting habitat for many other organisms such as burrowing owls, tiger salamanders, and hundreds of species of insects and spiders. Further, prairie dogs serve as prey for numerous predators – American badgers, bobcats, and coyotes to name a few mammalian enemies, and golden eagles, northern goshawks, and prairie falcons to name a few avian enemies. Escaping extinction for at least one species, the black-footed ferret, is dependent on the long-term survival of prairie dogs, because ferrets feed almost exclusively on prairie dogs. If prairie dogs disappear, grassland ecosystems as we know them will vanish as well.

John hopes that his research will help to conserve prairie dogs. One of his key findings is helping to dispel a century-old myth perpetuated by farmers and ranchers: that prairie dogs are fast-breeding like mice and rats, and will take over grasslands unless they are exterminated. By contrast, John's tracking of thousands of prairie dogs first captured as juveniles demonstrates that prairie dogs do not live long (usually only 1 or 2 years), that many females do not reproduce every year, and that successful females can only wean a small number of offspring (usually three or four, with a maximum of eight) per year.

One-year-old female white-tailed prairie dog that has just killed a juvenile Wyoming ground squirrel via a series of bites to the neck. Photo by John Hoogland.

John has just begun to write his 'magnum opus' – a book in which he will attempt to summarise and explain all 44 years of his research with prairie dogs. He reflects, 'If we want to save prairie dogs and their ecosystems, knowledge is power, and my research is providing information on every aspect of the biology of prairie dogs. Unfortunately, I will not live long enough to answer all the questions I still have.

I especially want to better understand the serial killing of juveniles of close kin by female black-tailed prairie dogs, and the serial killing of Wyoming ground squirrels by white-tailed prairie dogs.'

Meet the researcher

Professor John L. Hoogland
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Professor John Hoogland completed his BSc (1971) and PhD (1977) at the University of Michigan. After completing a postdoc at the University of Minnesota in 1979 and serving as an Assistant Professor at Princeton University for several years, John joined the Appalachian Laboratory of the University of Maryland Center for Environmental Science in 1985, where he is currently a Professor of Wildlife Ecology. Since he first saw them when he was a graduate student in 1974, prairie dogs have fascinated John. Over the last 44 years, he has devoted his career to finding answers to questions about the conservation, ecology, and social behaviour of these amazing grassland rodents. As John frequently says: 'Gotta love those prairie dogs.'

Awards during John's career include the Peter Okkelberg Award for outstanding achievement in biology (1971), the American Society of Mammalogists Alma Shadle Award (1976), the Harry Frank Guggenheim Career Development Award (1986), and the Prairie Dog Protector of the Year Award (2015). Citations to John's research number >5,000, and span >120 journals. His discoveries have been reported in hundreds of newspapers around the world, 10 textbooks, 9 radio programs, 11 television programs, and also >50 popular magazines such as Atlantic, Audubon, National Geographic, National Wildlife, New Scientist, Ranger Rick, Science News, and Smithsonian.

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Since he started studying prairie dogs in 1974, 22 different organisations have funded John's research. Recent sources of funding include Colorado Parks and Wildlife, Denver Zoological Foundation, National Science Foundation, and New Mexico Department of Game and Fish.

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BATS IN TRANSLATION

Dr Mirjam Knörnschild and her group at the Free University Berlin study the acoustic communication and social behaviour of wild bats to understand the evolutionary interplay of social and vocal complexity, the functional significance of culturally transmitted song dialects, and the importance of social learning processes for vocal repertoire ontogeny and flexible foraging behaviours.

Social and Vocal Complexity

According to the Machiavellian intelligence hypothesis, the driving force in the evolution of the human intellect was social expertise, which enabled our ancestors to cooperate with social group members or to manipulate them. Since social interactions require communication, the complexity of vocal communication signals should be tightly linked to the complexity of the respective social system. This positive feedback loop between social and vocal complexity has been investigated in primates, rodents, mongooses and certain birds. Bats, however, have not been thoroughly studied with regard to their communicative abilities, despite the fact that their taxon is extremely species-rich, comprising over 1,300 species, and that they exhibit a wide range of different social structures and mating systems. Moreover, their sophisticated echolocation behaviour is a prerequisite for a versatile vocal communication system.
Dr Mirjam Knörnschild and her research group at the Free University Berlin in Germany work with wild bats to understand the evolutionary interplay of social and vocal complexity. 'We study how, what and why bats communicate with conspecifics,' Dr Knörnschild states. 'In particular, we focus on one family, the Emballonurids or sac-winged bats.' This insectivorous bat family occurs in the tropics and subtropics of both the Old and the New World. One member, the greater sac-winged bat *Saccopteryx bilineata*, is one of the most thoroughly studied bat species worldwide. *Saccopteryx bilineata* has a complex social organisation. Related males compete for access to females by defending adjacent roosting territories in perennial day-roost colonies. Subordinate males queue for territory access in their birth colony and participate in defending their colony against unrelated immigrant males. Females base their mate-choice decisions on multimodal male courtship displays and seem to evaluate male quality year-round. This intense sexual selection pressure has led to elaborate male vocalisations used for repelling rivals and attracting mates. The level of social and vocal complexity observed in *Saccopteryx bilineata* is in stark contrast to the comparatively simple vocalisations of socially monogamous members of the sac-winged bat family, which Dr Knörnschild's group also studies. While intermediate levels of social and vocal complexity can be found in various species of sac-winged bats as well, *Saccopteryx bilineata* certainly represents the far end of the complexity continuum. No other member of this bat family possesses a similarly sized vocal repertoire, acquired partly though vocal learning and containing some of the most elaborate male songs found in bats to date.

Culturally Transmitted Song Dialects

Male bats of several species sing just like birds do, even though most of the songs' sound energy is concentrated in the ultrasonic range and thus not audible to humans. Bat song, just like bird song, contains information about male identity

‘I am especially intrigued by the versatile acoustic communication system of bats and how deciphering bats’ vocalisations allows us to get glimpses into their cognitive abilities and social relationships’

and quality. Moreover, the syllable structure and phonological syntax of bat song can be as rich and complex as bird song. Scientists have known since the 1970s that some bats sing, but only recently the ultrasonic recording equipment became robust and portable enough to conduct in-depth field studies on singing bats in the wild. ‘Studying bat communication in the wild is still a fairly new field that is rapidly advancing,’ Dr Knörnschild explains. ‘It’s really exciting to be part of this scientific community.’

Male *Saccopteryx bilineata* sing mainly at dusk and dawn while perching in their territories. In contrast to many birds, these bats sing year-round even though singing is most intense during the mating season. One song type, the territorial song, is directed mainly at male rivals in the vicinity, while the other song type, the courtship song, is used to address individual females roosting in a male’s territory. Territorial songs are especially well suited for playback experiments because males readily engage in counter-singing activities with real and virtual opponents. Dr Knörnschild’s group found evidence that males encode aggressive intentions in the pitch of territorial songs (a lower pitch indicates a more serious dispute than a higher pitch), that the daily song rate of males is influenced by the number of rivals to repel and females to guard (males sing more when they have more to lose) and that singing at dawn not only repels rivals but also facilitates the immigration of new females into existing colonies. The latter finding indicates that *Saccopteryx*’ territorial songs, just like the majority of bird songs, has the dual function of rival deterrence and mate attraction. But the similarity between bird song and bat song does not end at their function. Even their acquisition process is similar. Comparable to oscine songbirds, the territorial song of *Saccopteryx bilineata* is learned by imitating the song of a tutor male. Throughout ontogeny, bat pups listen daily to male songsters in their vicinity and learn how to sing. Pups imitate the tutors’ songs precisely but not perfectly, and the combined weight of small copying errors and subtle social modifications of song structure leads to the existence of distinct regional song dialects. These song dialects are culturally transmitted, passed on to the next bat generation through vocal imitation. ‘Since territorial songs are sexually selected signals, regional song dialects could function as reproductive barriers between different populations,’ Dr Knörnschild emphasises. ‘We are currently studying how the cultural transmission of song dialects could facilitate speciation.’ Female *Saccopteryx bilineata*, which leave the social group they were born in as sub-adults, rely on male territorial songs to find new colonies. It is thus conceivable that culturally transmitted song dialects constitute dispersal barriers for females, limiting gene flow between adjacent populations. Cultural transmission in animals is widely viewed as a potent ‘second inheritance system’ complementing genetics. The regional dialects in *Saccopteryx bilineata*’ songs seem to fit right in.

Bats for Biolinguistics

Bats’ ability for vocal imitation is by far not the only reason why their ontogenetic development is interesting. *Saccopteryx bilineata* pups babble conspicuously during vocal ontogeny, producing long strings of vocalisations in which elements from the adult vocal repertoire are repeated, juxtaposed and modified. This juvenile behaviour, sometimes also called vocal play, is reminiscent of the canonical babbling found in human infants. Babbling

is considered to be crucial for mastering the phonological challenges of vocal repertoire acquisition in different species, including humans. However, babbling behaviour is severely under-studied in non-human mammals. Apart from humans, *Saccopteryx bilineata* is the only known mammal so far that is both a babbler and a versatile vocal imitator, making it extremely interesting for biolinguistic studies.

The newly emerging field of biolinguistics tries to understand how and why the human language faculty evolved, an endeavour of exceeding difficulty. From a biolinguistic perspective, the crucial components of human language (e.g. vocal imitation, syntax, semantics, etc.) share the general properties of other biological systems and should thus be studied using a comparative approach. A fundamental question in this context is which aspects of language are uniquely human and which homologous or analogous traits can be found in animal vocalisations. Bats are a highly promising group for biolinguistic studies. Their ability to navigate and hunt via echolocation is a preadaptation for sophisticated vocal communication, and researchers are just beginning to understand the underlying complexity of the social vocalisations of bats. Moreover, many bats are gregarious and long-lived, which gives individuals ample opportunities to learn from conspecifics. Dr Knörnschild's group is convinced that studying bats will yield valuable insights into human language evolution, on both proximate and ultimate levels. 'Our aim is to establish bats in biolinguistic research,' Dr Knörnschild says. 'In our studies, we are combining state-of-the-art methods from behavioural ecology and (neuro-)genetics. Eventually, this will help us to better understand which preadaptations contributed to the evolution of the human language faculty.'

Social Influences on Foraging Behaviour

Dr Knörnschild's research is by far not limited to bat acoustics. Another focus of her group is the acquisition of novel foraging behaviours through social influences. To do this, Dr Knörnschild's group studies the foraging behaviour of wild Neotropical bats that feed on fruits or nectar. While individual learning certainly plays a huge role in optimising the foraging efficiency of bats and is intensely studied because of it, social influences on bats' foraging behaviour have received much less attention so far. Social learning happens whenever individuals acquire knowledge by interacting with others or observing them from a distance. Since frugivorous and nectarivorous bats roost gregariously and feed on clumped resources in the forest (i.e. fruiting or flowering plants), they have many opportunities to interact with conspecifics at their roosts or their foraging grounds. 'We investigate whether wild bats acquire novel foraging preferences or techniques by interacting with experienced conspecifics and how this new knowledge spreads in their social group,' Dr Knörnschild says. 'This offers the exciting possibility to study the cultural transmission of novel foraging behaviours in the wild.' Moreover, Dr Knörnschild's group studies various other important aspects of socially influenced foraging strategies, namely the temporal defence of food patches by dominant individuals, the recruitment of conspecifics to valuable food sources, and vertical learning of foraging behaviour from mother to offspring.

Future Directions

Dr Knörnschild plans to put more research emphasis on the cognitive accomplishments of bats in the future. Specifically, Dr Knörnschild's group will study how individual variation in cognition is related to consistent among-individual differences in behaviour (i.e. personality). It is conceivable that proactive bats, which are bold and rapid explorers, are faster but less accurate in cognitive tasks than reactive bats, but the latter might be better suited to deal with changes in their environment. Moreover, Dr Knörnschild's group will investigate the influence of sleep, or the lack thereof, on the consolidation of newly acquired information in bats' long-term memory. Sleep deprivation should interfere with acquiring new information and with remembering it later, no matter whether the information has been gained through individual or social learning. Furthermore, Dr Knörnschild will conduct interactive playback experiments with wild bats to study whether bats are capable of multimodal recognition of individual group members and to which extent bats have knowledge about third-party relationships between conspecifics. These findings will help assessing whether bats possess a 'theory of mind', i.e. whether they can attribute mental states – attention, intents, knowledge, etc. – to oneself and others, which would allow them to explain and predict the behaviour of conspecifics. 'I am convinced that we, as a scientific community, have only just tapped the surface of what bats are capable of,' Dr Knörnschild says. 'Learning more about their communicative and cognitive abilities will be especially advantageous for comparative studies, because bats are such a speciose and highly diverse mammalian taxon.'

Meet the researcher

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Dr Mirjam Knörnschild achieved her PhD in Zoology at the University of Erlangen-Nuremberg in Germany under the supervision of Otto von Helversen. Afterwards, Dr Knörnschild held a 5-year position as a postdoctoral researcher at the Institute of Experimental Ecology at Ulm University, working in the labs of Elisabeth Kalko and Simone Sommer. Since 2015, Dr Knörnschild is a Heisenberg Fellow leading an independent research group at the Free University Berlin in Germany and a Research Associate of the Smithsonian Tropical Research Institute in Panama. Dr Knörnschild's research group studies communication and cognition in bats, focusing mainly on wild bats in Costa Rica, Panama and South Africa.

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PHOTO CREDITS

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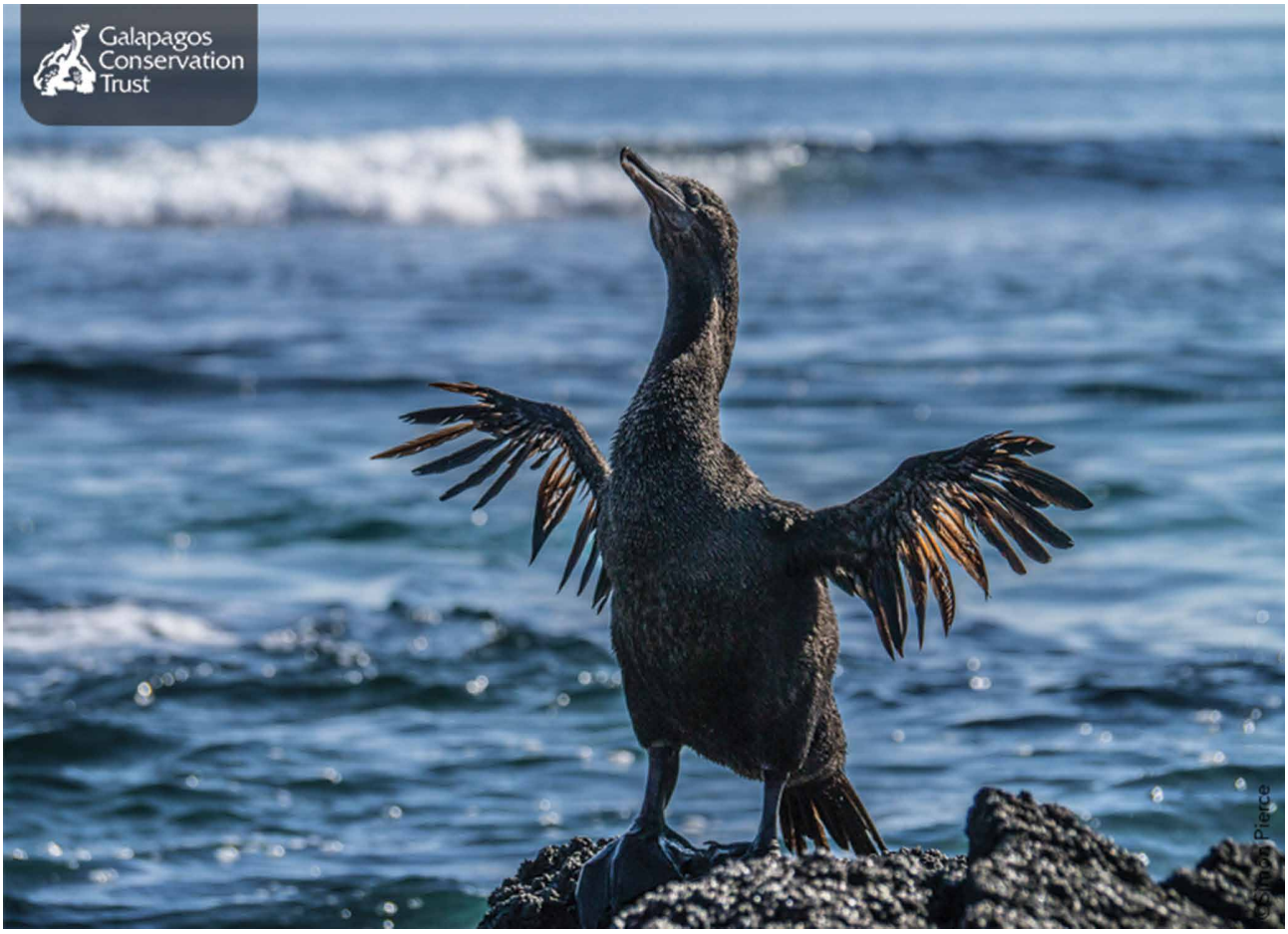
Foraging *Carollia perspicillata.* Credit: Michael Stifter
Foraging *Lonchophylla robusta*. Credit: Marco Tschapka
Dr Knörnschild recording bats. Credit: Claudia Rahlmeier
Saccopteryx bilineata mother and pup. Credit: Michael Stifter
Dr Knörnschild holding a bat. Credit: Claudia Rahlmeier

Smithsonian Tropical Research Institute

Freie Universität Berlin

THE GALAPAGOS CONSERVATION TRUST



Galapagos Conservation Trust (GCT) is the only UK registered charity to focus exclusively on the conservation and sustainability of the Galapagos Archipelago. Launched in 1995 at the Royal Society, GCT has supported a vast array of projects in Galapagos focussing on science and conservation, as well as sustainability and education. GCT's mission is to support, develop and promote projects that achieve measurable conservation, sustainable living, and protection of the environment. In this exclusive interview, we have had the pleasure of speaking with Jenny Vidler and Sharon Johnson of GCT, who tell us about their commitment to protecting the unique ecosystems of the Galapagos Islands.

The Galapagos Islands are home to a huge diversity of wildlife species, many of which are found nowhere else on Earth. What are the biggest threats that these unique species currently face?

The biggest threats facing the flora, fauna and marine life of Galapagos include invasive species, climate change and urban development. However, there are many other contributing factors that GCT is currently trying to fight. Man-made local issues such as human-wildlife conflict, and global issues such as marine pollution affect the health of the flora and fauna of Galapagos year-round. El Niño is also a pressing issue for vulnerable species across the archipelago, as the weather phenomenon is increasing in severity year on year due to climate change. Many species of wildlife fall in numbers, especially the Galapagos fur seal. The warming of the ocean reduces the food availability for the seals, causing starvation. During the 1982–1983 El Niño event almost all young fur seals were lost, with about 30% of adult females and non-territorial males, as well as almost 100% of the large territorial males.

Galapagos giant tortoises, made famous by Charles Darwin, are the largest land reptiles on Earth, and may live for over 150 years. Please tell us about your work in conserving these majestic creatures.

GCT is currently supporting the Galapagos Tortoise Movement Ecology Project (GTMEP). This project has a predominant focus on working with local people, especially farmers, to try to lessen the animal-human conflict in the highlands of Santa Cruz. This conflict between the world's largest reptile and local farmers is caused by the tortoises entering farms in the highlands of Santa Cruz and eating the farmers' crops. This issue is a serious problem for farmers, as they often live day to day on their crops, so any plants ruined by the tortoises are costly to their livelihood.

Through this project we hope to lessen the conflict by creating interest in the tortoises by providing a mediator between the tortoises and the farmers, as well as running after-school clubs for children to learn more about the importance of the biodiversity in the Islands, and the role the giant tortoises play. Activities include tracking the giant tortoises' migration routes, monitoring nest sites and hatchling numbers and understanding why the fluctuations in the hatchling population occur. GCT's GTMEP is the first project to ever study the giant tortoise hatchlings with such focus. This project is conducted with the hope of understanding why the severe fluctuations in population occur and to measure the severity of the outcome of the loss of the hatchlings.

What seabirds native to the Galapagos Islands are most vulnerable to the effects of climate change, and why? What is the Galapagos Conservation Trust doing to prevent their decline?

The seabirds of Galapagos most vulnerable to climate change are the waved albatross, the Galapagos penguin and the flightless cormorant. These three seabirds are particularly susceptible to the effects of climate change, as rising sea temperatures affect the birds' food source. The small fish that make up many sea birds' diets are affected by increases in sea temperature, leading to a decline in food. This affects their nesting habits and offspring, and ultimately the overall population.

Another issue facing seabirds of Galapagos, and indeed all marine life is the global problem of marine plastics. Plastics dumped into the oceans

'The seabirds of Galapagos most vulnerable to climate change are the waved albatross, the Galapagos penguin and the flightless cormorant'

and waterways of the world make their way into the sea currents, which means the Humboldt, Panama and South Equatorial currents draw ocean plastics towards Galapagos, which seabirds often mistake for fish.

GCT is helping to combat the issue of declining seabird populations by funding research teams on Galapagos to undertake annual seabird surveys. These surveys aim to monitor the populations of each seabird species. This insight will help us to understand and address the increasing issue of climate change and marine pollution, and will be instrumental in the reports sent to the Galapagos National Park and the Ecuadorian government. This information will be put forward in a bid to increase the legislation towards the protection of the local and global environment.

Although many Galapagos species are endangered, remarkably none have become extinct since before Darwin's time. However, only about 100 individual mangrove finches exist on the islands today, meaning that they are at high-risk of extinction. Please tell us why the population has become so low, and describe the ways that you have been working to boost numbers.

The two key factors causing the population decline of the mangrove finch *Camarhynchus heliobates* are parasitism of the chicks by the *Philornis downsi* fly, and the loss of their key mangrove habitat. The parasitic fly is an invasive species, introduced to the Islands by human settlement. The fly lays its eggs in the nests of the mangrove finch, and when the larvae hatch, the maggots feed on the blood of the newly hatching mangrove finch chicks. This fly is incredibly hard to eradicate due to a number of complex reasons. In order to combat the chicks falling fatal to the larvae, GCT supports a project which is aiming to lessen the impact this damaging parasite has on the mangrove finch. The Mangrove Finch Project head-starts the chicks, by collecting the newly laid eggs from the mangrove finch nests and transporting the eggs to a hatching facility on Santa Cruz, where the hatchlings are hand-reared until they are strong enough to be returned back to their

native habitat where they will survive on their own, avoiding the risks from *Philornis downsi*.

Another problem the finch faces is the loss of its natural habitat, the mangroves of Isabela. This is due to invasive plant species, though the removal of the mangroves by humans for the benefit of urban development is also a problem. GCT is working with the local population to help find sustainable solutions to the need for the expansion of the urban areas.

Increasing visitors and the growing human population pose some threats to the fragile ecosystem of the Galapagos Islands. Please explain some of the ways that you are engaging with local communities to ensure sustainability.

One of the greatest threats to biodiversity is the introduction of invasive species. 80% of the loss of biodiversity worldwide has occurred on islands where people introduced invasive species like rats, cats and goats, that devastate unique flora and fauna. Humans have introduced species both inadvertedly as we have travelled, and on purpose, and have devastated the islands' ecosystems.

GCT is currently working in partnership with a number of other organisations on a project with the aim to restore Floreana island back to its former ecological glory. Floreana island is an island steeped in history, both ecological and human. The native Floreana mockingbird inspired Charles Darwin's 'theory of evolution'; however, due to decades of invasive species damaging the native flora and fauna across the island, the Floreana mockingbird now only resides on two tiny nearby Islets.

The aim of GCT's Floreana project is to create a sustainable future for both the native wildlife and the local community. This is the largest project of its kind ever to be undertaken, and we are hoping to improve the island's biodiversity and ultimately bring the Floreana mockingbird back to its original home on Floreana island. This project is a new approach to community engagement and eco-tourism and we hope it will have a long lasting positive impact on the island. The iconic *Opuntia* cactus has also been strongly affected by the invasive species and this project hopes to reverse the damage done by restoring thousands of *Opuntia* across Floreana.

We know island ecosystems do recover if invasive species are removed, but this has never been managed on large human inhabited island before. This project is a unique opportunity to demonstrate we can achieve this in Galapagos. Fifty-five endemic species are threatened with extinction on Floreana due to out of control rat and feral cat populations. Working with Island Conservation, we are supporting new approaches to community engagement, including a range of eco-tourism activities – which will mean Floreana is poised to become the world's first large populated island to have its ecosystem restored, and the methods used will provide a template for archipelagos elsewhere around the world, benefitting hundreds of other threatened species. This project has already led to UN level recognition that Floreana can be the blueprint for the rest of the world's islands. Fifty-five endemic species will benefit and thirteen locally extinct species will be reintroduced.

Another element of sustainability GCT encourages is using less plastic across the archipelago. In the near future GCT will be launching a project to combat both marine and terrestrial plastic pollution with the aim to make Galapagos 100% sustainable and eco-friendly.

Finally, please tell us about the Galapagos Conservation Trust's focus on education, and its role in ensuring conservation efforts are maintained into the future.

GCT has been working on the educational website **discoveringgalapagos.org.uk**, a bilingual, interactive website focussing on the wildlife across the archipelago. Lesson plans are available for teachers to use for free as well as interactive games, videos, worksheets and interesting images. We believe that today's school students are tomorrow's conservation ambassadors and the future stewards of our planet. By deepening their understanding of the natural world and the conservation challenges that we face in the future, we hope to inspire and engage students in the sustainable development of Galapagos and the wider world through the Discovering Galapagos programme.

The isolation, iconic wildlife and unique geography of Galapagos have created a natural system with unrivalled subject matter for this kind of resource. The issues faced in the microcosm of Galapagos are of global relevance and the communication of these problems and their potential solutions have undoubted value for other areas of the world.

GCT also supports awareness raising outreach activities across Galapagos with a range of partners in order to provide a lasting message of conservation and sustainability to the local population. We support field trips for local students and youth groups to visit science projects such as the giant tortoise hatchling study on Santa Cruz to benefit from the experience of witnessing conservation first-hand. We also support community-led events such as the annual Shark Day held on San Cristobal island that draws attention to the value of sharks within Galapagos waters; the day aims to draw attention to marine conservation issues more generally using sharks as flagship species.

If you would like to learn more about Galapagos Conservation Trust visit **galapagosconservationtrust.org.uk**. Please donate to help GCT to continue ground-breaking science and conservation projects, and to preserve the unique flora and fauna of the world's most famous Archipelago.

PROTECTING AND RESTORING LIFE ON EARTH

At this stage in the issue, we are beginning to appreciate the origins of life on our planet and the explosion of life thereafter, leading to a multitude of different species. We have also touched on some of the very real threats to biodiversity and even the survival and success of our own species. These threats include climate change – the single greatest challenge of our times.

The ecosystems all around us are amazing in their complexity and diversity, but each exist in a delicate balance, consisting of a series of interdependent processes and species. It just takes one of these processes to change to upset this balance, completely disrupting the makeup and sustainability of an ecosystem. One devastating consequence of this disruption is species decline. In fact, human activity has already sparked a mass extinction – termed the Anthropocene extinction – with species becoming extinct at a rate estimated between 100 and 1000 times the normal background rate. However, all is not lost, in large part because of the efforts of conservationists to influence public opinion and policy, not to mention directly undertaking conservation efforts.

In this section of the issue, we pay homage to those scientists who are trying to conserve the ecosystems and creatures under threat from pollution, habitat loss and climate change. In the first article in this section we interview Heather DeCaluwe, Interim Executive Director, and Nathan Spillman, Marketing & Communications Manager of the Society for Conservation Biology. They discuss the Earth's rapidly declining biodiversity, and how the Society for Conservation Biology is working to restore and maintain life on Earth, through informing policy, supporting conservation scientists, disseminating research and encouraging collaboration.

Next, we introduce a team of scientists who are helping to bring the white abalone – a species of sea snail – back from the brink of extinction. Numbers of this edible marine gastropod have declined by over 99%, primarily due to overfishing. In order to restore populations, Drs Kristin Aquilino and Gary Cherr, along with their team at UC Davis, are working hard to breed these endangered critters in captivity, with the aim of reintroducing them back into the wild. Also working to conserve wild animal populations is Dr Scott Carver at the University of Tasmania. Dr Carver carries out research into the conservation and health of wildlife and ecosystems, with a particular focus on infectious diseases. In this section, we describe his wide-ranging research portfolio, which has recently included reducing sarcoptic mange in wombats and efforts towards developing a vaccine for chlamydia in koalas.

Next, we introduce the research of Dr Steve Greco at UC Davis, who develops sustainable solutions to boost our Earth's declining biodiversity. His team of landscape ecologists use spatial analysis and visualisation techniques to improve our understanding of

ecosystems and inform conservation efforts. In one particular project, his team used spatial analysis methods to identify suitable habitats for reintroducing the tule elk – a species that was brought to the edge of extinction in the 1870s, and for which reintroduction programs have had limited success thus far. They hope that their modelling approaches will improve the survival rate of the tule elk, and other reintroduced species.

To safeguard Earth's ecosystems into the future, we must focus on increasing conservation efforts not just on publicly-owned lands, but on privately-owned lands too. This involves engaging with landowners and gaining an understanding of the factors that motivate and encourage them to cooperate in conservation efforts. This is the area of expertise of Dr Urs Kreuter and his team at Texas A&M University, whose research into land use-related social sciences and ecological economics has been helping to shape how communities care for their local environments across the globe. Also working to increase conservation on privately-owned land is Dr Peggy Petrzelka and her colleagues at Utah State University. Partnering with the American Farmland Trust, Dr Petrzelka's team explores the social dynamics that female landlords face, and develops programs empowering land owners to drive conservation efforts on their land.

Of course, not all private land is owned by individuals – with a significant portion of the Earth's land owned by corporations, such as forestry companies. Forestry can act as a valuable habitat for many species and also plays a vital role in capturing carbon dioxide from the atmosphere. To improve the sustainability of the forestry industry, Dr René Germain and his team at SUNY ESF carry out research and outreach programs to build knowledge and explore better forest management practices. In this section, we explore some of their most successful endeavours to date.

From here, we move on to the topic of reducing environmental pollution and tackling waste. Our next article introduces the work of Dr Irina Druzhinina and her team at TU Wien in Vienna, Austria. She and her team are searching for compounds that can biodegrade plastic waste material, in order to reduce environmental pollution. In fact, they think such a substance may have already been invented – by a naturally occurring fungus. Using these naturally occurring compounds could be a neat way to clean up ecosystems polluted by human activity.

Last but not least, we shift our focus to improving methods of treating wastewater. Here, we showcase the innovative research of Dr Pascale Champagne and her environmental engineering group at Queen's University, who investigate the role of algae and microbes in wastewater treatment ponds. By exploring how these organisms respond to temperature and sunlight, the team is developing a modern, eco-friendly model for treating wastewater in Canadian climates.

THE SOCIETY FOR CONSERVATION BIOLOGY

The Society for Conservation Biology (SCB) was founded in 1985, when many scientists felt called to action by the Earth's rapidly disappearing biodiversity. Scientists saw their study sites changing or disappearing over very short periods of time, and were driven to protect what they could of the ecosystems they had come to know and love. By working together, they believed that they could avert what they saw as the worst biological disaster in the last 65 million years – one that would reach a crescendo in the first half of the 21st century. SCB was therefore established to bring together and give a voice to a global community of conservation professionals dedicated to preserving life on Earth. SCB envisions that such a global community can collectively avert this looming crisis through sound science practices. In addition to scientists, the Society's 5000+ members also include resource managers, educators, government and private conservation workers and students. This legacy informs SCB's mission, which is to advance the science and practice of conserving Earth's biological diversity. SCB achieves this by disseminating research through their publications, by supporting the next generation of conservation leaders through fellowships, training, and outreach, and by providing opportunities for conservation researchers and practitioners to share their work, form exciting collaborations, and influence policy and management decisions that affect Earth's biodiversity.

In this exclusive interview, we have had the pleasure of speaking with Heather DeCaluwe, SCB's Interim Executive Director, and Nathan Spillman, the Society's Marketing & Communications Manager. Here, they discuss the Earth's rapidly declining biodiversity, and how SCB is working to restore and maintain life on Earth, through informing policy, supporting conservation scientists, disseminating research, encouraging collaboration, and much more.

‘In addition to connecting a global community of conservation researchers and practitioners, one of our most important roles is to support, nurture, and inspire the next generation of conservation leaders’

Please tell our readers about the devastating decline in biodiversity that we are currently witnessing, as a result of human activity.

There is no way to sugar-coat the big picture: the continual growth of human population and our associated use of natural resources is unsustainable for the Earth. We see this manifest, in real time, in many ways – including climate change, the decline and extinction of many species, and the degradation of local ecosystems, including both our air and water quality. Moreover, because all life on Earth forms an interdependent web, the diversity loss we’ve already suffered imperils other at-risk species. As such, conservation of Earth’s resources has never been more important if we are to persist as a species. Moreover, time is of the essence, as we don’t know exactly how long we have to turn this ship around, so to speak. The Earth is, of course, an incredibly large system – the problems we face are the result of decades of action (and inaction), and the solutions will likewise probably require a significant period of sustained effort.

That said, there are plenty of successes and encouraging signs for the future of conservation biology. One of the most encouraging signs of the past decade has been the degree to which a large swath of the public is aware, interested, and cares about the environment in general and conservation biology, specifically. Further, when I look at our members, I do not see pessimism or defeatism – I see a group of motivated, excited, energetic, and talented researchers and practitioners. Within the context of our Society, they are passionate about their work and ideas, and excited to share with and learn from others in the field. Even with the challenges at large, I definitely see a lot of reasons for optimism.

Do you believe that we, as humans, have an individual and collective responsibility to restore and maintain biological diversity on Earth?

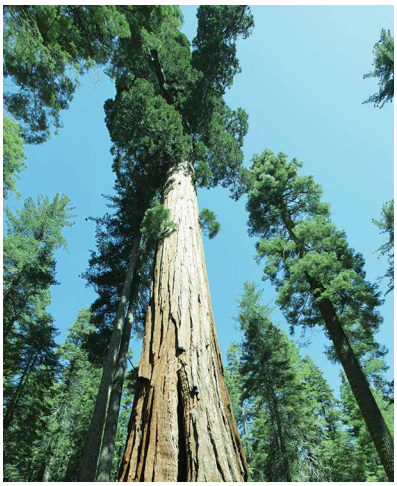
Absolutely – the responsibility we have as humans to maintain biodiversity is driven by both moral and financial concerns, as well as by basic survival. As humans, our actions have an incredible and in some cases devastating impact on the natural world around us. We are one of the estimated 8.7 million species inhabiting earth. By altering the earth’s climate and ecosystems, our actions influence nearly every other living species on earth today. Not only do I believe we have a moral obligation to protect the many other species on earth that we’re harming, but if humans want to survive as a species, we must take actions to preserve the biodiversity around us. For example, humans face a looming food crisis should we allow threatened bee and other pollinator species to go extinct. There are also financial reasons for preserving biodiversity, the so-called ‘ecosystem services’. For example, protecting forest ecosystems provides all animals on earth with oxygen to breathe, reduces climate altering carbon dioxide, helps protect watersheds, regenerates soils and aids in nutrient cycling, controls pathogens and provides medicines, and has a general cooling effect on the Earth. The financial impact of these combined benefits is valued at trillions of dollars. In addition to the moral obligation to preserve biodiversity on Earth, ignoring these direct benefits from ecosystem preservation is also severely short-sighted.

This obligation is felt by individuals, but the response to the challenges we face must be collective, in nature. To quote Michael Soulé, one of the founders of SCB:

‘We assume implicitly that science and technology are neither inherently good nor evil, but are tools that can benefit or harm. We assume implicitly that environmental wounds inflicted by… humans and destructive technologies can be treated by wiser humans and by wholesome technologies. Although we have varying personal philosophies, we share a faith in ourselves, as a species and as individuals, that we are equal to the challenge.’

SCB envisions a world where all people understand, value, and conserve the diversity of life on Earth, and where we hold one another responsible for the stewardship of our shared home.

‘The Earth is, of course, an incredibly large system – the problems we face are the result of decades of action (and inaction), and the solutions will likewise probably require a significant period of sustained effort’



How does SCB support and facilitate the creation and dissemination of conservation science? In what ways does this aid the management and conservation of ecosystems?

SCB publishes two scientific journals, *Conservation Biology*, which we founded in 1987 shortly after the formation of SCB, and *Conservation Letters.* The journals define the field of conservation biology by publishing ground-breaking research. They provide a space for scientists to debate emerging topics such as the role of economic development and poverty alleviation in conservation.

SCB also organises the International Congress for Conservation Biology (ICCB) every two years – to be held in Cartagena, Colombia this July. ICCB is recognised as the most important international meeting for conservation professionals and students. The Congresses connect our global community of conservation professionals, providing a forum for addressing conservation challenges. They are *the* global venue for presenting and discussing new research and developments in conservation science and practice. Further, they provide a venue for training early-career professionals, and for catalysing conservation action. ICCB is global in scope, bringing together conservation professionals and students from every sector of the field, including the biological and social sciences, management, policy and planning.

Moreover, the journals and congresses directly impact management decisions. Resource managers, educators, and other decision-makers look to the journal and attend our congresses to guide their actions on topics such as corridor design. Many papers that are published and presented through SCB translate science into policy recommendations and action items. In particular, *Conservation Letters* seeks to cover policy-relevant conservation research from the natural and social sciences.

How does SCB help to increase the application of science to policy?

Because conservation challenges vary greatly across the globe, so must our approach to influencing policy as a solution to said challenges. As a global Society, SCB is composed of seven regional sections – representing all six of the inhabitable continents plus the marine realm. Through our sections, we have a far more significant impact on the application of science to management, policy, and education.

One avenue for our sections to influence policy are by issuing resolutions, declarations, and policy statements. For example, our Asia Section puts significant effort into combatting the illegal wildlife trade. At its 2016 regional congress in Singapore, the Section issued a Resolution on Reducing Illegal Wildlife Trade and Trafficking in Asia. The Resolution offered seven recommendations to stakeholders in the region – including conservation groups, enforcement agencies, and legislative bodies – that will help to combat the trade in wildlife. Likewise, our Oceania Section issued a declaration on land clearing at its regional meeting in 2016 that called on governments to prevent the return of high rates of woodland and forest clearing to protect the unique biodiversity of its marine environments. Our Africa Section issued a position statement on the threat from expansion of palm oil plantations on biodiversity.

Sections can also influence policy through sustained action and collaboration with local governments. In recent years, our Europe Section urged leaders in Poland and beyond to reject changes to a Forest Management Plan that would allow for increased timber harvest in Bialowieza Forest. The Section has worked for many years to protect Bialowieza Forest and old-growth forest stands across the continent. The Society’s Latin America and Caribbean and Marine Sections have worked together in recent years to save the vaquita from extinction by writing to the Mexican government to ban gilnet fishing in vaquita habitat and our North America Section has worked to protect species under the Endangered Species Act, among other things.

Our regional sections provide an avenue for influencing policy in a way that harnesses the insights and understanding from those most familiar with the causes, effects, and societal ramifications for a diverse range of global conservation issues.

Tell us a bit about SCB's carbon offset project, and its benefits to biodiversity.

For more than ten years, SCB has contributed money to offset greenhouse gas emissions due to Society operations, including our biennial International Congress for Conservation Biology (ICCB). We've invested in a nature preserve in South Africa to restore sensitive native thicket habitat that supports rhinoceros, elephant and kudu. More recently, we've committed to a grasslands project in Alberta, Canada to eliminate grazing on a 390-ha cattle ranch. Grasslands are purported to store more carbon on a per area basis than any other ecosystem. The project also includes a biodiversity element as grasslands in southern Alberta are home to seven of nine endangered species in the province. In addition to selecting projects that will sequester carbon dioxide, SCB prioritises projects that benefit biodiversity, have long-term viability, and are certified. Currently, the Society is looking to invest in a new project to help offset greenhouse gas emissions from our upcoming ICCB in Cartagena, Colombia.

Through what means does SCB support students and early career scientists and conservationists?

In addition to connecting a global community of conservation researchers and practitioners, one of our most important roles is to support, nurture, and inspire the next generation of conservation leaders, at all stages of their education and early career. SCB manages the David H Smith Conservation Research Fellowship Program, which supports and develops future world leaders and conservation practitioners who are successful at linking conservation science and application.

SCB also supports students and early career scientists through our international and regional congresses, by offering trainings, student presentation and poster competitions and awards, resume-writing workshops, career fairs, and social and networking events that connect students and early career scientists with professionals in the field. Our congresses are organised to maximise their value for students and early career scientists. Poster sessions, for example, receive top billing and we offer travel reimbursement support to help students offset the cost of attendance.

Our journal *Conservation Biology* publishes papers on the conservation field that cover topics like how to succeed in the conservation job market and necessary skills for non-academic conservation careers. It also recognises papers by students and early career professionals with annual 'Rising Star' awards.

Additionally, we host an online Job Board and Career Center, provide leadership, planning, and networking opportunities through our local chapters (which are primarily student-run), and this year initiated our first Graduate Student Research Fellowship Awards. The program offers ten $1,000 fellowships to graduate students to support their field work.

Finally, explain the importance of a diverse, equitable, and inclusive community in restoring and maintaining biological diversity on Earth. How is SCB helping to work towards cultivating such a community?

Today's complex biodiversity conservation challenges require input and analysis from a variety of voices, vantage points, and expertise from different geographies, backgrounds, disciplines, and dimensions. A variety of viewpoints results in better ideas that can compete with and inform one another to find best solutions to conserve and restore biodiversity.

The success of conservation biology is intertwined with and relies on a commitment to greater access, inclusion, and empowerment of humans of many views, vantage points, identities and geographies who actively participate in conservation with equal opportunity and access at all levels of the Society's structure. Because successful conservation efforts rely to a large extent on the communities that live near and interact with sensitive ecosystems around the globe, incorporating the insights, ideas, and concerns from a diverse array of stakeholders at every stage of the process is essential.

The goal of the Society's Equity, Inclusion, and Diversity Committee is to engage a broader community of students and professionals in SCB's activities, and to help the Society embrace its potential to become more inclusive and welcoming of diversity broadly, and to foster a shared vision and accepting culture for the development of a diverse membership and leadership.

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Wild white abalone. CREDIT: Athena Maguire

BRINGING WHITE ABALONE BACK FROM THE BRINK

The white abalone snail has been overfished to the verge of extinction, but **Dr Kristin Aquilino and Professor Gary Cherr** at UC Davis hope to save the species by reintroducing their captive-bred population back into the wild.

CREDIT: Sammy Tillery

Threatened by Overfishing

Although trying to get sea snails in the mood for love might not be everyone's cup of tea, Dr Kristin Aquilino loves her job at the frontline of building up captive breeding populations of the endangered white abalone. 'I love the sense of purpose that comes with restoring an endangered species, advancing knowledge, promoting science and education, and supporting sustainable aquaculture,' she tells Scientia. The white abalone (Haliotis sorenseni) was once common in kelp forests off the coast of Southern California and Mexico, and has been harvested by humans for around 10,000 years. It plays an important role in the kelp forest ecosystem, competing with other algal grazers such as sea urchins and keeping their numbers under control.

Valued as a delicacy, white abalone also historically supported a fishery, making an important contribution to the local economy. However, ever-increasing demand, coupled with newer fishing techniques, led to intense overfishing in the 1970s. This pushed the white abalone to the edge of extinction in California. At its most abundant sites, white abalone numbers dropped by around 80% in eight years, from an estimated 15,000 in 2002 to 3,000 in 2010, and to less than 0.1% of its historical, baseline level. After some earlier conservation measures, white abalone fishing was finally banned in 1996. Despite this, in 2001, the species had the dubious honour of becoming the first invertebrate on the US federal endangered species list.

Although the white abalone is also found in Mexican waters, the status of its population there is unknown, and it is thought to be in significant decline. Even with the fishing ban in place, the US population has yet to show signs of turning the corner. Only one juvenile white abalone has been found in the wild in the last 30 years. One of the obstacles affecting the snail's recovery is that, for it to breed, males and females need to be within a few metres of each other. Only when they are close together can the eggs released by the females be fertilised by the sperm from the males – and with the low numbers left in the wild, spawning individuals are too far apart. Scientists agree that a captive breeding program is the only hope of survival for the white abalone, and without such efforts, it could be extinct within 20 years.

Breeding White Abalone in Captivity

At the White Abalone Culture Facility in the Bodega Marine Laboratory (University of California, Davis), Dr Aquilino and Professor Gary Cherr carry out research into the white abalone's reproductive biology, hoping to ultimately improve the survival of captive-bred snails in the wild. Professor Cherr, a reproductive biologist, explains why he finds this particular research project so rewarding, 'The endangered white abalone captive breeding program was an opportunity to apply my background and interest in reproductive biology to a conservation-oriented project with the goal of restoring a species which almost completely disappeared because of human beings.'

The Bodega team is part of the White Abalone Recovery Program, a consortium of partners working together to save the white

‘By replacing overhead pipes with towering kelp forests and swapping out submersible pumps for steady ocean swells, we hope our precious baby snails might save their species from extinction’ – Dr Aquilino

Juvenile white abalone. CREDIT: Joshua Asel

abalone, with members from federal and state agencies, aquariums and the California aquaculture industry, as well as UC Davis and UC Santa Barbara. Thanks to funding from the NOAA National Marine Fisheries Service, their breeding program has experienced dramatic increases in success, producing 20 one-year-old abalone in 2012, 150 in 2013 and an impressive 10,000 in 2016. The captive population now almost certainly outnumbers the few remaining wild abalone.

The breeding program owes its success to the Bodega Marine Laboratory research team’s dedication to getting the conditions just right for each stage of the abalone’s complex reproductive cycle. Discussing the challenges, Professor Cherr says, ‘A single sperm cell must swim through treacherous waters to reach an egg, penetrate through the protective layers surrounding the female germ cell, and then successfully fertilise it. This is a truly remarkable feat and is a wonder of biology. For our work with the white abalone, we are trying to overcome all odds and not only enable high fertilisation rates for millions of eggs, but also for subsequent embryo development, larval hatching, and finally larval settlement and metamorphosis. This is not an easy task when every egg, embryo, and larva are as precious to the world, as they are with the white abalone.’

To get the captive abalone to breed, the scientists start by adding small amounts of hydrogen peroxide to the seawater, which introduces reactive oxygen species that encourage the abalone to spawn, releasing eggs and sperm. The team collects these and combines them in the best ratios for fertilisation. This produces embryos, which are transferred into a hatching container, and hatched larvae migrate into buckets through special tubes. When the larvae are old enough (~7 days), the team adds a biochemical signalling substance which induces them to settle on tiles covered with the small microalgae that they feed on. At around 5 months old, the juvenile abalone can be weaned on to larger leafy algae.

The White Abalone Recovery Program’s early breeding efforts were affected by outbreaks of a bacterial disease known as withering syndrome. The bacteria attack the abalone’s gut, preventing it from producing digestive enzymes, so it cannot feed and its muscle withers, and they may die. This killed almost all of the estimated 100,000 juvenile abalone produced in 2001 at a facility in Southern California. Dr James Moore (UC Davis School of Veterinary Medicine and California Department of Fish & Wildlife) has developed a strict hygiene regimen at all facilities. The abalone are kept in seawater which has been filtered and sterilised with ultraviolet radiation to kill the bacteria that can cause withering syndrome. For when new white abalone come into captivity, or in rare cases when captive white abalone contract the bacteria, the team has developed an antibiotic bath treatment that eliminates the bacteria. Controlling the temperature of the abalone’s seawater is also important, as this disease only occurs at higher temperatures. Another possible cause of abalone death is damage by organisms such as polychaete

‘The endangered white abalone captive breeding program was an opportunity to apply my background and interest in reproductive biology to a conservation-oriented project with the goal of restoring a species which almost completely disappeared because of human activities’ – Professor Cherr

Close up of eggs being spawned out of a newly-collected wild female. CREDIT: Michael Ready

Adult white abalone. CREDIT: Kristin Aquilino

worms, which can bore through their shells. To keep their snails happy as clams, the team even wax the adults’ shells twice a year, protecting them from boring worms. ‘Between antibiotic cleansing baths and exfoliating, coconut oil and beeswax treatments, our white abalone healthcare plan now reads like a relaxing spa retreat,’ Dr Aquilino jokes.

The Road to Recovery

The program reached a special milestone in 2015, when it produced enough young abalone for Dr Aquilino, Professor Cherr and their team to distribute some to their partners. The main breeding program has now expanded to the small populations at five other institutions. Perhaps most exciting, the team is also making plans for how to reintroduce the white abalone into the wild. The Bodega Marine Laboratory scientists hope to transplant their first captive abalone within the next couple of years. To avoid risking the fragile captive white population, Dr Laura Rogers-Bennett’s team with the Bodega Marine Laboratory and the California Department of Fish and Wildlife are currently experimenting with a less threatened relative, the red abalone, to conduct outplanting trials. They are looking at ways to maximise the survival of their transplanted abalone, such as relocating sea stars and octopus from the reintroduction sites to reduce their predation of abalone, and stocking abalone in winter when some predators will be less abundant.

The team also hope to discover which sites offer the best conditions for abalone at different life stages, based on their understanding of these factors in captivity, and more research and monitoring of abalone and their preferred habitats in the wild. Some of their key questions include whether abalone prefer certain surface types, whether artificial reefs can provide suitable habitats, and which temperatures are most suitable. Dr Aquilino and Professor Cherr explain that it is also unknown whether sea currents and competition with other species affect wild abalone mortality, and whether environmental changes due to climate change will have an impact.

The team is keen to ensure that their captive population is as healthy as possible, so that they have better prospects of surviving their challenging transition to life in the wild. To that end, they would like to further refine their breeding and rearing techniques, to increase reproduction to minimise the mortality of young abalone. ‘We are looking forward to continuing to enhance our reproduction and rearing techniques through experiments aimed at understanding reproductive conditioning and survival during their early life stages,’ Dr Aquilino says.

One of the issues that concerns them about their captive breeding population is the possible negative effect of working with a relatively small number of individuals – low genetic diversity in the adults could lead to offspring with lower levels of fitness, and this becomes more likely with each generation of inbreeding. So the team applied for a special permit from the US National Oceanic and Atmospheric Administration, to allow them to take a small number of white abalone from the wild to supplement their breeding program. They were delighted to receive the permit in September 2016. The wild abalone they gather will introduce vital new genetic material into the captive population. One newly-collected female spawned in March 2017, marking the first time in 14 years that new genes had been added to the captive population.

The team also plans to use some exciting new techniques to increase the genetic diversity. ‘We have started to freeze (cryopreserve) sperm from a number of male white abalone and these frozen sperm cells can be stored for years but thawed as needed for fertilising eggs from females with different genetic backgrounds,’ Professor Cherr explains. ‘This can allow us to create genetic crosses that previously were not possible with the use of only fresh sperm. Our goal is to increase the efficiency of both in vitro fertilisation methods as well as embryo and larval culture techniques such that we can increase the percentage of juvenile white abalone in our breeding facility in Bodega Bay, CA. This will accelerate the timeline for our imminent release of young white abalone back into the field.’

Thanks to all the hard work of the Bodega Marine Laboratory research team and their partners, the white abalone could be one of the great success stories of conservation. Dr Aquilino is optimistic about the snail’s future: ‘By replacing overhead pipes with towering kelp forests and swapping out submersible pumps for steady ocean swells, we hope our precious baby snails might save their species from extinction.’

Meet the researchers

Dr Kristin M Aquilino
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Dr Kristin Aquilino was awarded her BSc in Zoology at the University of Wisconsin in 2005, followed by her PhD in Population Biology at the University of California, Davis in 2011. She continued her work at UC Davis as a postdoctoral scholar from 2011 to 2013 in the Bodega Marine Laboratory, after which she worked at NOAA National Marine Fisheries Service as an independent contractor from 2013 to 2016. Since 2016, she has been Assistant Project Scientist at the UC Davis Bodega Marine Laboratory. She recently received the 'Species in the Spotlight Hero Award' from the US National Oceanic and Atmospheric Administration for her work trying to save white abalone.

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Professor Gary Cherr
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Professor Gary Cherr obtained his BA in Biology from Sonoma State University in 1979, and his PhD in Zoology from the University of California, Davis in 1984. From 1984 to 1986 he was a National Institutes of Health Postdoctoral Fellow at the UC Davis School of Medicine. In 1986 he joined the UC Davis Bodega Marine Laboratory, and in 1999 he became Professor in Environmental Toxicology and Nutrition at the Laboratory. He was Acting Director from 2007 to 2008, Interim Director from 2009–2015, and Director from 2016–present.

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KEY COLLABORATORS

NOAA National Marine Fisheries Service
California Department of Fish and Wildlife
Aquarium of the Pacific
Cabrillo Marine Aquarium
Santa Barbara Museum of Natural History Sea Center
University of California, Santa Barbara
The Cultured Abalone Farm

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CONSERVATION, CONNECTIONS OF HUMANS AND NATURE, AND UNRAVELLING A WORLD OF INFECTIOUS DISEASES

Dr Scott Carver and his team at the University of Tasmania use a multidisciplinary approach to study the spread of disease within and among wild animals, their domestic counterparts, and humans.

'No man is an island,' says the poem, 'entire of himself.' It was intended to remind us of the many connections we hold to those around us – our friends, neighbours, and acquaintances. But there are other networks which surround us, just as important and yet often overlooked. These are ecological connections we have to the natural world through the food we eat, the air we breathe, the environment we live in.

These connections are brought sharply into focus by infectious diseases. Recent rises in wildlife pathogens causing global headlines of disease in humans (Ebola, swine flu and SARS for example) and vice-versa for wildlife (canine distemper virus, amphibian chytrid fungus, bat white nose syndrome) highlight our intimate connections with the natural world. For example, a virus moving from a rodent, a bat, a sick bird, or a pig, can adapt to a human, who can infect others, who may travel and spread the infection to new locations. Suddenly you have a runaway epidemic causing quarantine zones across the world. The small-scale connections we have with our local ecology have a major effect on our global society, and vice-versa.

Wombat-Scale

So let's take a step back and focus on the small scale. These headline problems often overshadow the less-known yet vitally important problems occurring via the spread of disease in local animals. A little-known yet devastating example of this is currently occurring in Australian wombats. Wombats – cute, ground-dwelling, chunky marsupials with big noses – can be found in burrows scattered across the south-eastern parts of Australia. Their populations are currently under attack by sarcoptic mange – a disease caused by the *S. scabiei* mite – which is widespread across their populations. Sarcoptic mange is a serious disease which, if left untreated, almost inevitably leads to death of the host – and indeed is currently killing large numbers of wombats. Similar problems are seen in other native Australian animals – Koalas are currently heavily affected by chlamydia infections which lead to blindness, infertility, and death, while transmissible facial tumours are currently decimating the Tasmanian devil population.

It is at this point that Dr Scott Carver and his team at the University of Tasmania enter the picture. Dr Carver's work is multidisciplinary, covering a number of fields of research. 'Fundamentally,' he commented, 'I am interested in the conservation and health of wildlife and ecosystems, and also in the connections between humans and their domestic animals to the natural world. I have a relatively broad research portfolio, with key systems: disease transmission in puma and bobcats; sarcoptic mange in wombats; chlamydia in koala and agricultural animals; and mosquito-borne disease transmission.' Dr Carver has taken an international road to his current role, starting out in New Zealand with a focus on a fungal disease threat to amphibians. He then moved across to the University of Western Australia where he gained his PhD studying the ecology of the Ross River virus. The virus, a mosquito-borne disease, leads to severe arthritic joint pain and chronic fatigue-like symptoms in humans. The virus naturally cycles between marsupials and mosquitoes, spreading to humans under particular circumstances. We asked Dr Carver for more details on his early work. 'As an early graduate student,' he replied, 'I became fascinated by how pathogens could spread from one species to another – termed "spill-over". This has important health and population consequences for humans, domestic animals and wildlife and, in my eyes, shows the intimate connections between humans and the natural environment. This shaped the direction of my research, spanning health and conservation. As I progressed in my career I also grew to love the discovery of new knowledge associated with science.'

This focus on invasive diseases and their human/animal interaction continued with his

‘I saw the dramatic impacts invasive diseases could have on wildlife populations and this shaped the direction of my research’

move to the University of Montana, where he began to study the Sin Nombre hantavirus. Sin Nombre (the ‘nameless virus’) is found in native mice found across almost all of the United States, and it can jump across to humans who inhale dust in buildings contaminated with virally infected mouse faeces and urine. The viral infection leads to flu-like symptoms and a pulmonary oedema in humans – an inability to breathe which kills a third of those infected. Dr Carver’s work used the ecology and epidemiology of the mouse and virus to improve knowledge of disease outbreaks and environmental factors underlying transmission. This research was able to show that a combination of arid environments and seasonal changes in mouse behaviour are likely significant factors for human infection during Sin Nombre virus outbreaks.

Wild Cats and Viral Loads

Another move brought him to the University of Colorado, where Dr Carver examined cross-species exposure and transmission between domestic cats and their wild counterparts – bobcats and pumas. With several publications on both feline disease transmission and on prion diseases in wild deer populations, his success was able to set the stage for much of his current and future collaborations. In 2014 he and several other researchers were awarded a prestigious $2 million grant from the US National Science Foundation with the goal of understanding how environmental factors and wildlife management programs affect the spread of infectious disease in puma (also known as cougar, mountain lion and panther) and also bobcats. Building upon their previous work involving extensive field sampling across multiple areas of the US, the group uses cutting edge host and viral genomics and mathematical modelling to examine how disease spreads through animal populations, and how this spread is affected by actions such as hunting, animal relocation, habitat fragmentation and urbanisation. Wide-ranging species such as pumas are of particular interest to researchers due to their pivotal ecological roles as apex predators.

The team’s previous research – examining a suite of different pathogens among puma, bobcats and domestic cats – has shown that transmission between species is relatively rare. Pathogens rarely make the jump from domestic to wild animals or vice-versa. However, once that jump has occurred and the pathogen is able to gain a foothold in the new host species, then the spread within the new host species can be self-sustaining. While this research may appear far removed from humans, the reality is that animal diseases are far more relevant to human health than we like to believe, with spill-over of disease between species acting as a major contributor to human health. We need look no further than recent high profile examples such as Ebola, SARS, Hendra virus and pandemic influenza.

This research led to other subjects, as it so often does, and Dr Carver’s work then moved beyond specific pathogens to the general processes by which pathogens spread amongst and between groups. Research performed with his collaborators led to the finding that the majority of newly discovered pathogens in wildlife are due to exposure to humans, domesticated animals or invasive animals introduced by humans. This was observed worldwide in fish, amphibians, birds, and mammals.

Bound for Van Diemen’s Land

Dr Carver returned to Australia in 2012, taking up a position at the University of Tasmania in the School of Biological Sciences. His research interests in Ross River virus and feline diseases were quickly joined by further interests covering diseases affecting marsupials and other Australian native animals (primarily wombat mange, koala chlamydia and Tasmanian devil facial tumour disease). While everyone is aware of the role infection plays in our normal lives (we’ve all experienced the flu, after all), the effects of disease on wild animals are often

underestimated and poorly understood. This is partly because of a lack of research into the field and also due to the sheer complexity of the problem. As Dr Carver comments, 'pathogens can have such pervasive impacts in the natural world, yet their impacts are often enigmatic – requiring real creativity to unravel and influence.'

The approaches used by Dr Carver's group are as varied as the problems they study. They perform field studies on pathogens and host organisms, and field and laboratory experiments to understand disease impacts and transmission, and use mathematical modelling to determine the underlying processes. In the course of doing this they seek to answer questions, such as – how exactly do you know where a disease outbreak has come from, how quickly it will spread, and what factors will affect the growth of the outbreak? If a cure or treatment can be developed, where should it be targeted for maximum impact, and how can we support it? Complicating this is the simple fact that the real world is messy, data is difficult to come by and has missing pieces – answering these questions requires 'real creativity'.

This ecological and epidemiological approach can be seen in the team's efforts to combat the pathogenic disease, sarcoptic mange (caused by a skin burrowing mite), which affects wombats across their range. The mites in question, known as *Sarcoptes scabiei,* feed on skin cells, blood and serum by burrowing into the skin. Adult mites mate on the skin surface and then the females tunnel into the skin, laying eggs as they burrow. The larvae hatch, burrow out of the skin, before burrowing into hair follicles where they pupate into nymphal and adult stages. The entire process takes two to three weeks and leads to very severe symptoms, including irritation, hair loss, thickening and cracking of the skin and loss of body condition – the wombats themselves rapidly become emaciated and weak. Without treatment, an affected wombat will die, slowly and painfully. The mite was likely introduced to Australia by European settlers and their domestic animals – *S. scabiei* is found across the world in species as diverse as dogs, cats, buffalo and humans. From there the mite spilled over into Australian wildlife, with new variants developing for the multitude of new host species.

'Mange is the most important disease of wombats,' comments Dr Carver, 'and it is capable of causing dramatic population declines. It also occasionally affects other marsupials in Australia, including koalas.' His team works with the community, government and volunteers as field researchers, keeping track of wombat health and determining the spread of parasitic mange, as well as testing ways to control it. This is coupled with studies on the physiological and immune responses of wombats to mange infection, as well as studies to characterise the genomes of both wombats and the mange mite. The intention is to better understand why mange affects wombats so badly and how it can best be controlled.

This expertise is also part of a concerted effort to understand the impacts of chlamydia in koalas and develop a vaccine against it. *Chlamydia pecorum* is endemic amongst many wild koala populations, where it can lead to infertility, blindness and death. It also occurs in agricultural animals, from which it may spill over to koalas. Treatment of koalas requires doses of antibiotics that are generally not possible to deliver to wild animals. As such, vaccines represent an important option for dealing with this disease, and so part of Dr Carver's epidemiological research is to support the development of therapeutics for wild koala populations.

Tracing Networks

So, where to from here? We asked Dr Carver about his future research plans. 'Increasingly,' he replied, 'my research is moving toward investigating mechanisms of disease transmission and control in wildlife populations. This is undertaken using computer simulations and field experiments. This work also involves close collaboration with molecular biologists and veterinarians.'

As can easily be seen, Dr Carver's work is both multidisciplinary and broad in scope. His research touches upon diseases in wild animals, in domestic animals, in humans, and the many ways in which they can spread from one to another. This research gains ever more importance as human impacts on the environment continue to spread further into previously wild areas, leading to increased contact with wild species and thus higher chances of new diseases and pathogenic spread. Through their work on the spread of disease, Dr Carver and his team are trying to sustain a better future for the fascinating yet fragile wildlife of Australia, while supporting domestic animal and human health.

Meet the researcher

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Australia

Dr Scott Carver began his scientific career in New Zealand with a focus on amphibian disease. He subsequently continued to pursue and expand his interests, becoming a successful researcher in the field of wildlife ecology and epidemiology. He has over 60 publications to his name of which almost 20 were published in the last two years. His research group and collaborations are funded through a number of agencies to the value of several million dollars. This research has been the subject of numerous television and radio programmes, and online and written articles.

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RECENT NOTABLE PUBLICATIONS

S Carver, SN Bevins, MR Lappin, EE Boydston, LM Lyren, M Alldredge, KA Logan, LL Sweanor, SP Riley, LE Serieys, RN Fisher, TW Vickers, W Boyce, R Mcbride, MC Cunningham, M Jennings, J Lewis, T Lunn, KR Crooks, S Vandewoude, Pathogen exposure varies widely among sympatric populations of wild and domestic felids across the United States, Ecological Applications, 2016, 26, 367–381.

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LANDSCAPE ECOLOGY: THE SENSE OF SPACE

Developing sustainable solutions to environmental decline will need a holistic understanding of socioecological connections in time and space. **Professor Steven Greco** and his team at UC Davis use spatial analysis and visualisation techniques, to improve our understanding of ecosystems, and to inform conservation efforts.

Scale in Conservation

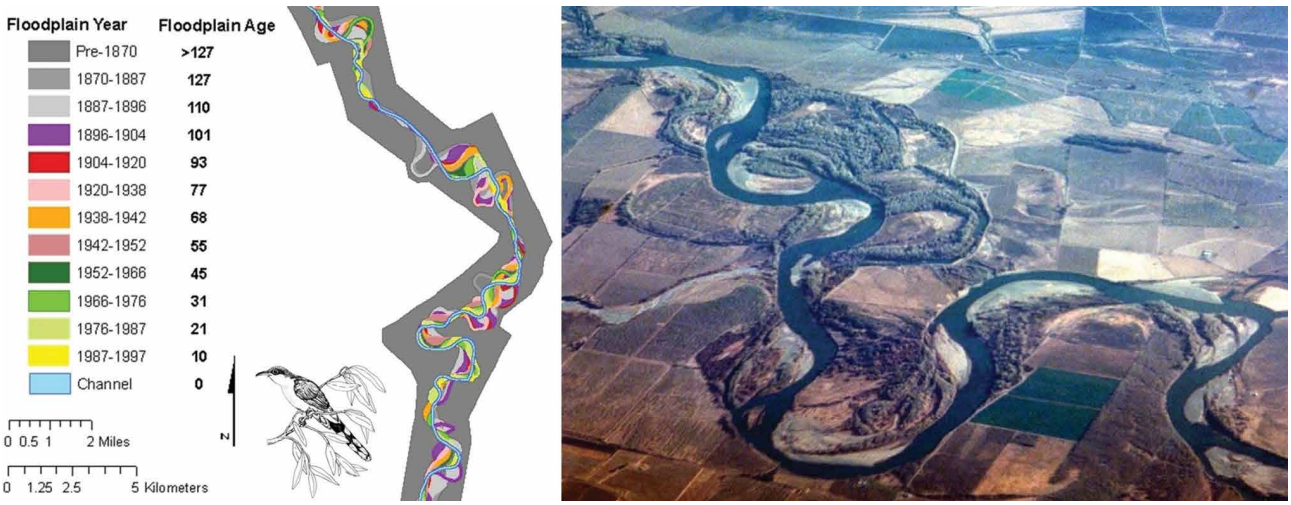
All living organisms exist in a complex web of connections, embedded in the context of the broader ecosystem. As human populations continue to increase, placing natural land under increasing pressure, understanding these connections will make a crucial contribution to preserving and restoring ecosystem function, and to the success of any conservation programme.

Landscape ecology is an approach that focuses on the interacting ecological processes that take place at different spatial scales. These scales range from local mechanisms such as genetic exchange, to regional and inter-continental ones such as disturbance or migration. Professor Steven Greco, a landscape ecologist at UC Davis, describes how his work informs conservation. 'My research aims to understand how natural systems function in cultural (human-created) landscapes at a variety of spatial and temporal scales and how to inform ecological design to improve those systems,' he explains.

Scale is often overlooked in approaches to conservation planning, although it is vital to consider its effects. Global conservation effort often focuses on ecoregions – areas with similar physical geography and characteristic patterns of biodiversity. However, land use planning is typically carried out at the local level, which limits the legal structures available for implementing regional-scale conservation plans. In a study published in 2010, Professor Greco and his colleagues explored the role of spatial scale in the design of conservation networks in the California Central Valley ecoregion. They found that the choice of a local or regional scale led to dramatic differences in the areas identified for inclusion within a conservation network. Only around a third of the average area at either scale was identified at both scales. Connectivity, considered important to allow the free movement of animal populations between sites, was also notably affected by planning scale. The team also found that just 43.8% of local corridors overlapped with regional ones, while just 53.5% of regional corridors overlapped with local ones. Both planning scales led to the loss of important information for conservation planning: local planning alone resulted in fragmented networks, while regional planning overlooked locally important areas. Achieving the best possible outcomes for conservation will require the use of approaches that integrate information from both local and regional scales.

River Dynamics Aid Natural Succession Processes

Riparian, or river-bank, zones are an example of an ecosystem whose management and restoration requires well-targeted planning. Riparian systems depend on the ability of the river to move and create new habitats through erosion and sediment deposition. These changes allow the river system to support different successional stages of vegetation. In California, riparian zones have the greatest biodiversity of any ecosystem. Human activity can restrict the natural development of riparian ecosystem changes by modifying river flows through dams, or by artificially reducing bank erosion. Planning for the restoration of degraded riparian ecosystems requires an understanding of the ways in which rivers meander, and how this affects vegetation patterns. One way to do this is by producing maps of the age of floodplain surfaces.



CREDIT: photo: Marli Miller (marlimillerphoto.com); illustration: Caroline Hipkiss

Professor Greco and his colleagues used the Sacramento River in California to explore the ways in which riparian ecosystems are affected by land age. The Sacramento River is the largest river in California, and its active meandering constantly produces new land. This allows primary succession by willow and cottonwood trees, providing habitat for threatened wildlife such as the western yellow-billed cuckoo. However, an estimated 95% of the river's riparian forests have been lost through conversion of the land to agriculture and urban uses, and through flood control measures such as the Shasta Dam. In their study, published in 2007, Professor Greco and his colleagues developed a method of overlaying time slices of floodplain maps to track the patterns of Sacramento River floodplain area over time. They used aerial photographs to map the riparian community vegetation, and link the distribution to the floodplain age class. They found that 71% of riparian vegetation was on land less than 101 years old, and willow and cottonwood had the highest proportional cover on land aged 1–9 and 10–44 years respectively, emphasising the importance of natural river meandering for these habitats. The team's approach, which used an automated geographic information system (GIS) environment, was designed as a tool that could be used by land managers to assess the reference conditions at sites, in terms of the proportions of different vegetation classes. This would provide a useful baseline to aim for in designing restoration plans, or to minimise the impacts of construction projects.

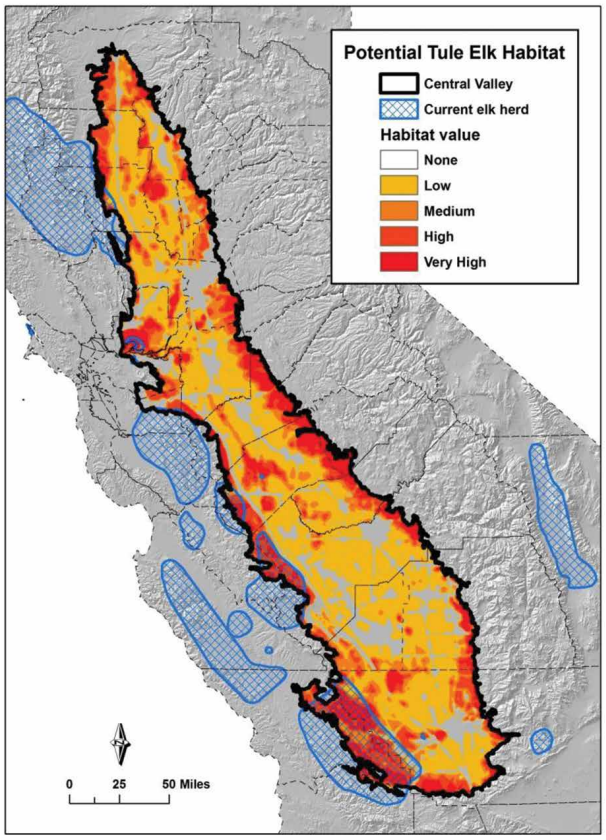
The role of floodplain age is one of many factors that should be taken into account in the design of landscape projects such as open channels for flood control. Typical channel systems are designed solely for the purpose of flood protection, and are narrow, deep and smooth to maximise water flow and minimise their land footprint. However, these features also reduce their potential as riparian habitats. Professor Greco and his colleagues showed that redesigning flood control channels can enhance their ecological value. In a study published in 2014, the team simulated the effects of expanding the channel footprint area in the Sacramento River region. The river's antiquated flood control system is currently being redesigned, offering opportunities to improve its role in conservation. The team simulated scenarios with wider channels, and calculated the maximum roughness coefficients – corresponding to higher levels of riparian vegetation – which would achieve the target water flow rates and hence flood protection levels. In all three areas of the river examined, it was possible to increase the roughness coefficient and attain improved flood protection, although this would require a greater land area for the wider channels. In the case of the Sutter Bypass region, it would be possible to design a channel capable of supporting dense woody riparian vegetation.

The western yellow-billed cuckoo, mentioned above, is an endangered subspecies in California whose riparian habitats are affected by changes in the patterns of river meandering. This cuckoo favours cottonwood habitats around the Sacramento River, dynamic patches of forest whose distribution is constantly in flux, depending on the meandering of the river. Professor Greco has been involved in several studies on yellow-billed cuckoo habitat in the region, one of which explored how the distribution of cottonwood forest on the Sacramento River changed between 1952 and 1987. He used GIS maps of land cover derived from aerial photographs to quantify the amount of woody riparian vegetation in 1952 and 1987. Building on the method used in his 2007 floodplain study, his team further identified subpatches of vegetation on young floodplains as likely cuckoo habitat.

The results, published in 2013, showed that 16% of the river's riparian vegetation was lost in the 35 years between the two measurements, likely through conversion to agricultural land. Only 15% of the patches in 1952 had the same location in 1987, providing evidence of rapid changes in distribution. One positive finding was that the significant reduction in new floodplain land produced was partially offset by a higher rate of colonisation by cottonwood, probably due to reduced erosion as a result of the effect of Shasta Dam in decreasing water flow. This led to the comparatively small drop of 11% in cottonwood forest production. The new patches were mostly formed through oxbow or floodplain lake processes, highlighting the particular importance of these processes for riparian habitat conservation. Maintaining a stable mosaic of cottonwood patches will require a management approach that allows natural river meandering processes, if populations of the yellow-billed cuckoo, and other threatened riparian species, are to remain viable.

Spatial Modelling to Bring Back the Tule Elk

Another native species in California which could benefit from the broad-scale landscape approach to conservation is the tule elk.



Final habitat suitability values for tule elk in the Central Valley ecoregion

CREDIT: Jason Huang

This subspecies of elk is endemic to California, but hunting and the large-scale habitat loss to agricultural land development brought it to the edge of extinction in the 1870s, with approximately 2–4 individuals remaining (based on genetic evidence). Captive breeding programmes and reintroduction efforts have increased their numbers to an estimated 3900, although there are no free-ranging animals in the Central Valley portion of their former range. The success of reintroduction has been hampered by unanticipated effects, some of which might be reduced through systematic, spatially explicit habitat analyses to identify optimal release sites.

In 2011, Professor Greco and his colleagues used spatial analysis methods to produce the first systematic evaluation of potential habitat for tule elk reintroduction. Their work evaluated the Merced County Grasslands Ecological Area in the Central Valley, based on the estimated spatial values of land cover and forage, habitat diversity and human impacts. These variables were combined in a geospatial model, with a final suitability value as output, based on the geometric mean of the three input values. The team identified two large areas of high-quality habitat in the San Luis National Wildlife Refuge, which would be suitable candidate sites for release: the San Luis and East Bear Creek Units, and the Kesterton Unit.

In a subsequent study, Professor Greco and his colleagues used a new spatially explicit population model, HexSim, to assess the potential of four different sites in the Central Valley for tule elk release. HexSim offers improved realism through simulation of animal herd movements and barriers to movement. This allows for the incorporation of likely human-elk conflicts. The team used data from other elk herds in California and expert knowledge to add appropriate parameters to the model. Their work, published in 2014, found that the 25-year population forecast was highest at the East Bear Creek site, and this site also had the lowest number of conflicts. This confirmed the team's previous finding on the suitability of this site, and they hope that modelling approaches of this kind could improve the survival rate of reintroduced species.

Landscape Ecology for a Better Future

Professor Greco is also passionate about the need to equip new generations of ecologists with the tools to apply landscape approaches to conservation. He is the founder of the Landscape Analysis and Systems Research (LASR) Laboratory at UC Davis, currently hosting four graduate students and one Assistant Project Scientist. He also teaches a variety of courses, including site and landscape ecology and GIS for mapping environmental features. One of his recent teaching projects was a case study actively involving students in the design of ecological greenways, using wildlife habitat relationship (WHR) models to produce functional habitat areas for selected focal species. Although WHR models allow landscape architects to incorporate essential information on species life histories, they are not yet taught in most programmes. Professor Greco's work demonstrates the utility of the concept in producing ecologically effective designs at the undergraduate level.

His team is now applying their experience of landscape ecology techniques to contribute to broad-scale conservation planning efforts in California. In a new research project sponsored by the National Institute for Food and Agriculture through the Hatch Fund, they are carrying out research to enable the design of sustainable habitat conservation systems such as ecological networks at multiple scales. In this project, running from 2016 to 2021, they will continue their work on the development and implementation of spatial analysis and visualisation techniques, to improve our understanding of ecosystem structure, function and change. Their work will also focus on exploring the roles of novel ecosystems in cultural landscapes, and of hydrological systems such as the Sacramento River, to enable ecosystem service provision including flood control while delivering improved outcomes for conservation. Finally, Professor Greco sums up their plans: 'We intend to study impacted river systems to promote natural values and to provide access for people to experience natural systems through greenway planning and design in urban and agriculturally-dominated landscapes. Ultimately, I'd like to integrate the three modes of landscape conservation: protected reserve areas, ecologically restored areas, and reconciled areas. Protection and restoration of natural resources is greatly needed to improve and maintain environmental quality for both people and wildlife.'

Meet the researcher

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Professor Steven Greco obtained his BSc in Landscape Architecture in 1987 from the University of California, Davis. During his work as a post-graduate researcher, he was awarded his MSc in 1993, and then his PhD in Ecology from UC Davis in 1999. From 2000 to 2007, he worked there as Assistant Professor, and from 2007 he was Associate Professor, before being promoted to full Professor in 2015. From 2015 to 2017 he was Vice Chair of the Department of Human Ecology and Chair of the Landscape Architecture and Environmental Design Program. He is also the founder of the Landscape Analysis and Systems Research Laboratory. Professor Greco teaches courses on a variety of different topics, including Site Ecology, GIS, Landscape Ecology, and Conservation Planning.

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COOPERATION, CONSERVATION AND FIRE: ENGAGING PEOPLE IN ECOSYSTEM MANAGEMENT

In the worldwide effort to protect the resilience of diverse ecosystems, privately owned lands are becoming of increasing importance for conservation efforts. Effective management of these lands for wildlife and the continued delivery of critical ecosystem services often involves collaboration between multiple landowners across large areas. **Professor Urs Kreuter** of Texas A&M University recognises that to safeguard habitats and ecosystems for the future, it is necessary to understand the factors that motivate and encourage landowners to cooperate in these efforts. His work in land use-related social sciences and ecological economics is helping to shape how communities care for their local environments across the globe.

Growing up in southern Africa, Professor Kreuter developed a fascination for the diverse ecosystems of his childhood home. His strong interest in ecosystem biology and environmental conservation, coupled with the realisation that effective policy in these fields must be driven by understanding human behaviour, led him to a career studying the social and ecological economic aspects of ecosystem management. He realised that by studying the ways in which people are motivated to use and maintain their land by both government imposed policies and community based initiatives, he could help be a positive force for the application of land management practices that benefit conservation efforts for natural resources.

In many developed countries, privately held land encompasses more area than federally owned reserves. Much of this land is rural and holds potential to maintain both plant and animal biodiversity in healthy ecosystems if landowners are properly motivated and educated in environmentally friendly land management practices. His work helps governing bodies and community organisations identify barriers to effective land management and implement programs that are more successful in encouraging landowners to cooperate in the care of native ecosystems. 'My research focuses on understanding factors that encourage landowners to coordinate land management decisions at spatial scales that exceed their own landholdings,' Professor Kreuter explains. He is interested in what drives the owners of private land to collaborate with their neighbours for the greater good of local ecosystems.

Quantifying the Effects of Urban Sprawl

One of the major interests of ecological economists is quantifying the value of ecosystem services and tracking their loss due to urbanisation. Ecosystem services involve the benefits gained from properly functioning ecosystems that are essential to life on Earth, and they include air and water filtration, carbon sequestration, and nutrient cycling. Changes in land use can greatly impact the delivery of ecosystem services, but measuring such changes can be difficult due to large volumes of often-incomplete data. This is further complicated by difficulties in quantifying the difference between obvious human-caused changes, such as housing development, and more subtle changes, such as plant composition shifts due to the spread of invasive species. Professor Kreuter and his team sought to tackle this challenge using LANDSAT satellite

‘My research focuses on understanding factors that encourage landowners to coordinate land management decisions at spatial scales that exceed their own landholdings.’

images from the area surrounding San Antonio in Texas, USA – an area undergoing rapid growth in urban sprawl. They compared images from 1976, 1985, and 1991 and calculated changes in areas of rangeland, woodland, agricultural and urban land. They related these land cover types to three watersheds in Bexar county and estimated the ecosystem services that each cover type would provide. They found a 29% increase in urban land and a 65% decrease in rangeland, yet their initial calculations showed only a 4% decline in ecosystem services. Traditionally, forested land is weighted to offer the highest ecosystem services, and they realised that this minimal decline was likely due to woody plant expansion into rangeland over time. If rangeland and forest ecosystem services were held equal, the decline in services increased to 15.4%. This study highlights the need to track both habitat loss and habitat type when evaluating ecosystem services loss due to urban sprawl. Certain types of land cover, such as urban tree canopy cover, may mask the negative effects of urbanisation, but may also help mitigate these effects when planned and used purposefully.

Working Together for Wildlife Management

Another important area of inquiry in ecological economics is understanding how community structure impacts land and wildlife management on private land. This is particularly relevant to large animal management in areas with expanses of undeveloped private landownership. Large animals typically require contiguous areas of land to graze and roam; thus, their home ranges may extend over numerous properties. Hunting of these animals on private land must be carefully managed to ensure that their populations are healthy and remain sustainable for multiple generations. This presents a common dilemma, a situation in which short term human interests may be at odds with the long-term needs of the species. Professor Kreuter’s team is interested in the factors that encourage landowners to make decisions that benefit the greater good, both for their local landowner community and for the wild species that inhabit their land.
In predominantly private lands states, such as Texas, wildlife management associations (WMAs) have emerged as a mechanism for coordinating decisions regarding wildlife across multiple landholdings. The success of these associations depends on collaboration between landowners, which necessitates strong social capital that is determined by high levels of trust, reciprocity, and community involvement among WMA members. Professor Kreuter and his colleagues evaluated the influence of social capital on the function of four WMAs in Texas, USA, aimed at managing populations of white-tailed deer. Two of these WMAs were larger associations formed predominantly by newer landowners who met more often, held longer standing memberships in their WMA and exhibited lower levels of social capital. The other two WMAs had fewer members, who were predominantly male and owned larger properties that they primarily used for hunting rather than as a place to live, yet they exhibited stronger social capital.

Professor Kreuter’s team found the most influential factor positively affecting social capital was group size; memberships over about 30 appeared to lead to a decline in social capital. Other factors included greater length of property ownership, and greater frequency of WMA meetings. Negative factors included absentee land ownership and the proportion of woodland vs. rangeland on the property. By capitalising on the factors that

encourage social capital, and minimising the factors that discourage it, WMAs are more likely to develop sustainable cooperative wildlife management practices across larger landscapes.

In southern Africa, community based private land management is proving essential for wildlife conservation efforts. In the past decade, legislative changes have shifted authority over wildlife management from central government to private landowners, while the commercial value of wildlife has steadily increased. These factors have led to a great increase in the amount of private land dedicated to conservation, such that the area of privately-owned land devoted to wildlife is now more than double that of government protected areas. It has also become increasingly common for adjacent landowners to incorporate their properties into private nature reserves that have no internal boundary fences. This not only increases the contiguous land areas available for wildlife but also requires collaborative community-based management of these lands and pooled resources to promote conservation efforts.

Professor Kreuter's team examined the mechanics of three such private wildlife reserves in South Africa and Zimbabwe in an effort to understand the factors that contribute to effective community-based natural resource management on privately owned land. While all of the reserves exhibited characteristics needed to sustain collaboration, such as effective leadership and value of resources, factors such as tenure of landownership, number of coordinating owners, use of an overarching wildlife management entity, and support of the surrounding community varied among the three reserves, but may ultimately prove more important in the long term sustainability of these reserves.

Burning in Control

Much of Dr Kreuter's recent work has been devoted to challenging stigmas surrounding the controlled use of fire to maintain grassland and savannah ecosystems. Once viewed as natural disasters and horrible accidents, scientists now recognise that periodic fires are necessary for the proper functioning of fire-adapted grasslands and savannahs. In these ecosystems, native herbaceous plants depend on periodic burn events so as not to be overwhelmed by the expansion of fire-prone woody plants. Furthermore, when these landscapes experience regular smaller burns, there is less fuel load accumulation and, therefore, less risk of wildfires that start unintentionally and often burn out of control, consuming homes and property.

Historic fire frequencies in grasslands and savannahs can be replicated through a process called prescribed burns, where fire intensity and boundaries are carefully controlled such that only the intended land is affected by the fire. Prescribed burns help to clear encroaching invasive species and woody plants, benefiting native fire-adapted plants. This process allows grasslands to flourish following a fire, and helps protect the integrity of these increasingly threatened ecosystems. From Professor Kreuter's perspective as an ecological economist, prescribed fire also offers an opportunity to study community dynamics in resource management. 'The application of prescribed fire, which is necessary to maintain the functionality and productivity fire-driven ecosystems, is an ideal tool for enhancing collaboration and greater coordination of land management,' he explains.

The grasslands of the US Great Plains are a prime example of a fire-adapted ecosystem. Fire is critical to maintaining healthy grassland structure and composition, but is has been excluded for long periods

of time in many places. As a result, juniper trees have encroached on millions of acres of grassland, degrading these ecosystems and reducing the provision of many of their valuable ecosystem services. In the past decade, private citizens have banned together to form 'burn cooperatives' – groups that promote the use of fire as a tool for grassland management. Professor Kreuter and his colleagues have documented the positive effects of these cooperatives on local ecosystems and on shifting attitudes towards fire as a land management tool.

In areas with a burn cooperative, landowners are granted access to a dedicated social network that provides education and support for performing prescribed burns, and are more likely to take a positive view on prescribed burning in general. In Texas, such cooperatives are known as Prescribed Burn Associations (PBAs) and have emerged to restore ecosystems in areas with predominantly private landownership. PBAs offer education, training, equipment and labour to help landowners minimise risk when engaging in a large-scale burn. Landowners are more willing to use fire when limitations of skill, knowledge, and access to fire management resources are removed. Burn cooperatives empower citizens to enact change in their communities that benefits the grassland ecosystems they inhabit.

Professor Kreuter and his team have identified one of the greatest barriers to the use of prescribed burns as legal liability. This factor is most often identified by landowners as the reason they are not willing to use fire to maintain their land. Many state and local governments have begun to recognise the benefits of prescribed burns in maintaining healthy ecosystems and reducing the risk of uncontrolled wildfires, and to consider changes in legislation to promote rather than inhibit the use of controlled fire in land maintenance. Professor Kreuter and his team compared various laws to identify those that best promote the use of prescribed fire. Landowners in locations with gross negligence liability standards were more likely to use prescribed burns, compared to those with simple negligence laws. Applications of additional permitting and safety laws did not reduce the use of fire, indicating that lawmakers can promote responsible fire use without limiting the practice. Professor Kreuter's ongoing work seeks to continue to identify the social and legal barriers and opportunities to enhancing the use of prescribed fire, in an effort to encourage landowners to use this practice to maintain grassland ecosystems more broadly.

Meet the researcher

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Professor Kreuter is the Joan Negley Kelleher Professor in the Department of Ecosystem Science & Management at Texas A&M University. He obtained BS and MS degrees in Agriculture from the University of Kwa-Zulu Natal, South Africa, and MA and PhD degrees from Utah State University. Before moving to the USA, Professor Kreuter worked as a Research Scientist in the South African Department of Agriculture and as a lecturer at the University of Kwa-Zulu Natal. He then joined Texas A&M University as a faculty member in 1998. Professor Kreuter's research focuses on the human dimensions of ecosystem management with the aim of increasing coordinated land management on private rangelands to enhance the delivery of ecosystem services. He has published over 100 articles and has presented over 80 talks at national and international professional meetings. Professor Kreuter currently teaches an undergraduate capstone course on Ecosystem Management; a study abroad course on Biodiversity Conservation and Eco-tourism in Southern Africa; and a graduate course on Ecological Economics. His most recent awards include Association of Former Students' Society for Management Range Science Education Council Outstanding Undergraduate Teaching Award (2015), Society of Range Management Outstanding Achievement Award – Research/Academia (2014) and Distinguished Achievement Award – College Level Teaching (2013). Dr Kreuter was also a Distinguished Visiting Scientist with the Australian CSIRO in 2011.

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TEXAS A&M UNIVERSITY

SOLUTIONS TO MODERN CHALLENGES IN GENDER, AGRICULTURE AND CONSERVATION

In recent decades, farmland in the United States is increasingly cultivated by operators that rent the land, rather than the land owner. This shift holds major implications for land use dynamics, particularly in conservation practices used in farming, and this variation is most apparent when rented land is owned by women. **Professor Peggy Petrzelka's** research describes the social dynamics female landlords face, while developing programs to empower land owners to drive conservation efforts on their land. When she started partnering with American Farmland Trust (AFT) in 2013, she was able to expand this research and use her results to begin to change these social dynamics. AFT is a national conservation organisation dedicated to preserving the nation's farm and ranch lands and critical natural resources like soil and water.

The way that agricultural land is farmed often impacts the surrounding areas, and can have dramatic environmental and economic impacts on a community, particularly on local watersheds. Many practices that are environmentally friendly, such as planting cover crops and using conservation tillage techniques, are also beneficial to soil health and crop yields, but require long term planning and investment in the land being used.

The efforts of agricultural conservation programs have often concentrated on the long-term benefits of environmentally responsible land stewardship; however, a large portion of the agricultural land in the United States is now being cultivated by operators who rent, rather than own, the land they farm. Landlord-renter relationships often directly affect the way that land is farmed, and can often be at odds with conservation goals. Professor Peggy Petrzelka at Utah State University, along with her colleagues at AFT, studies these relationships with a particular focus on female agricultural landlords, in the hope of improving landlord-renter relationships for the benefit of the environment and community.

The Shifting Faces of Land Ownership

Nearly one third of the 911 million acres of agricultural land in the United States is owned by non-operating land owners, with numbers steadily rising. These agricultural landlords are increasingly absentee – not living on, or even near, the farmland that their renters work to earn a living. Many are also increasingly multiple generations removed from farming the land they own, with no first-hand knowledge of the ins and outs of working and maintaining agricultural land.

Many non-operating land owners are unaware of farming best practices to maintain soil health, and lack effective relationships with their operators to influence the farming techniques they use. While farmers are more likely to be aware of environmentally responsible land management practices, many are dissuaded from following them on rented land, due to the great monetary and time investments. This dynamic can pose a challenge to conservation programs aimed at reducing nutrient and soil sediment runoff from agricultural land, since landlords can be hard to contact and may hold little sway over farming practices, while their renters may be hard to convince to make soil conservation measures on land that they do not own.

In 2014, 37% of non-operating land owners were women, accounting for roughly 25% of the rented land ownership in the United States. Despite owning millions of acres of farmed land nationwide, female non-operating land owners are one of the least studied demographics in the field of agricultural land tenure, which seeks to understand the ways in which different people have rights to land and how these dynamics impact land use. Research beginning in the early 1990s identified women non-operating land owners as a unique and important subset of agricultural

‘Changes in land ownership pose social, environmental and economic challenges – for both the landowners and the farmers they rent to. My work focuses on attempting to overcome some of those challenges, resulting in more conservation done on farmland, and protecting the financial viability of all involved.’

landlords that had been largely overlooked in studies of land use dynamics. This research recognises that female landlords often face additional challenges when it comes to managing the land they own in a manner that is both economically beneficial and environmentally responsible. ‘My work focuses on attempting to overcome some of those challenges, resulting in more conservation done on farmland, and protecting the financial viability of all involved,’ Professor Petrzelka explains.

An Ongoing Struggle for Women Land Owners

In past decades, a major assumption held about the relationship between land owners and their operators was that land owners held all the power in determining how the land was farmed. However, more recent studies detailing the relationship between agricultural land owners and renters have shown, that despite holding ownership of the land, the majority of owners have little say in how their renters choose to manage the land. The most common relationship is one in which the renter holds the dominant hand, as they are often the ones on site, providing labour, and financing the supplies used.

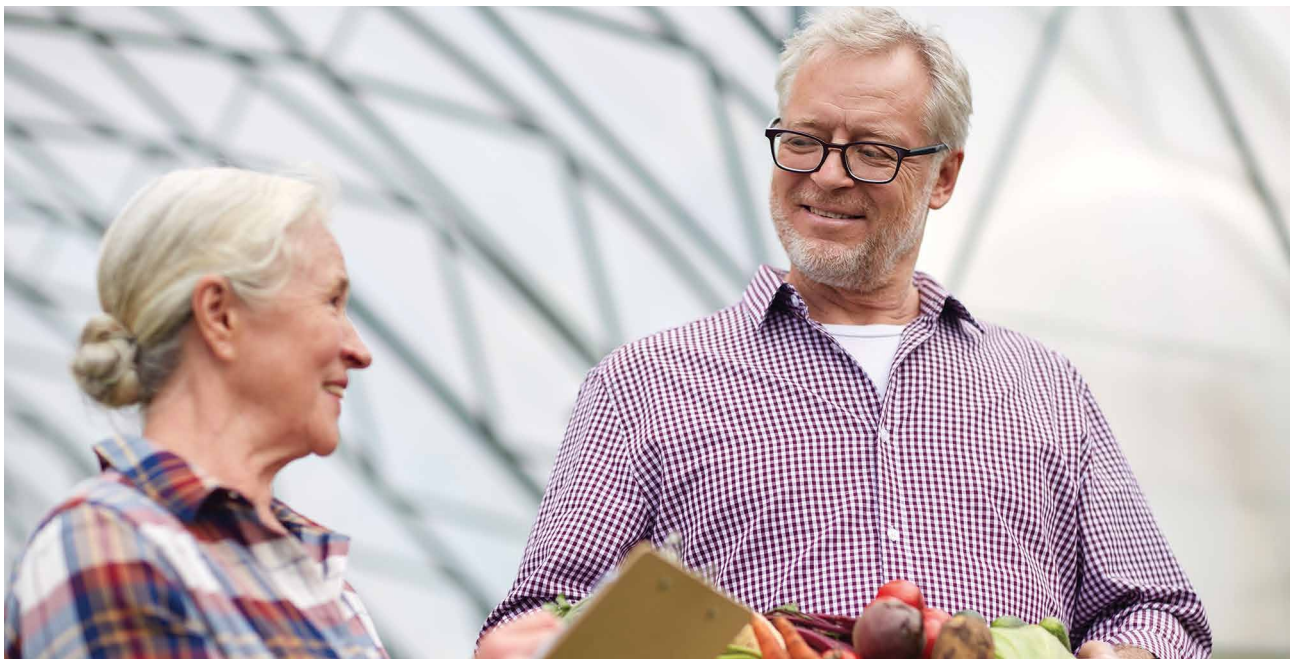
This is particularly true when the landlord does not live locally, is elderly and/or widowed, and even more so when the landlord is female.

Women non-operator land owners are more likely to have inherited their land and are frequently older than their male counterparts. They are less likely to live on the land, and less likely to have direct farming experience. They are more likely to engage in cash rent transactions, where the renter pays rent on the land and typically makes all management decisions, but also assumes all risk and reward, rather than a crop-share arrangement where the landlord and renter share management, risk, and rewards. As a result, female land owners are the least likely to share in management decisions or make land use directives to their renters.

Despite the large number of female non-operating land owners, very little research has been completed into the unique challenges they face in managing the land they own. These effects are at least partially related to encultured societal gender roles. Various studies of women non-operating land owners in Iowa in the early 2000s found that many female owners felt pressure to defer to their male renters in matters of land management. Many expressed a fear of ‘scaring away’ good renters, and all felt some degree of power inequity with their male renters, many expressing feelings of exclusion or alienation when it came to making decisions about their farmland.

Women land owners are more likely to comply with the directives of male renters in an effort to keep the peace, particularly when the renters are relatives. While female owners consistently show strong feelings of responsibility towards the community and are likely to support environmentally friendly farming practices, they are also more likely to be silenced by the patriarchal social structure in rural communities, which serves to place women landlords at a disadvantage.

Working with colleagues in Iowa and Michigan on absentee landownership specifically, Professor Petrzelka sought to further illuminate gender differences in non-operating land ownership, in cases when the owner was living in a different county than the farmland in question. They found that for both male and female absentee owners, land owners were more likely to be involved in decision making when they were younger



and when their renter was not a relative. However, female landlords' decision making was more heavily impacted by socioeconomic factors. Retired female owners were more likely to participate in decision making than women who had inherited land, or those that co-owned land with siblings. Women that relied more heavily on rent money for income were less likely to make decisions, regardless of age or relationship to their renter.

When looking specifically at conservation decision making, Professor Petrzelka and her colleagues found an even more striking trend. Male land owners were less likely to have a say in conservation practices only when they were not related to their renters. On the other hand, women land owners were significantly less likely to be involved in conservation practices in almost all situations, except when they co-owned land with a spouse. The team's research highlighted that the gender of an absentee land owner strongly influences whether or not they will have a say in how their land is used.

Encouraging Conservation

The imbalanced power dynamic between female land owners and their male renters poses a problem to conservation efforts. Female land owners are likely to appreciate agricultural conservation efforts that benefit the environment and community, yet they frequently have the least say in the farm management practices with the greatest environmental impact, such as fertiliser choice, crop rotation, use of cover crops, and tillage methods. Non-operating land owners in general are less likely to be involved with local farming extension programs and natural resource agencies – female owners even more so.

Despite expressing strong conservation values, many women landlords surveyed in the Midwest reported low confidence in their ability to implement environmentally responsible farming practices with their renters, and further reported feeling intimidated and ignored by agricultural agency staff when asking land management questions. Professor Petrzelka and her colleagues are engaged in efforts to shift agricultural agency practices to better support female land owners through both data collection and policy implementation. Programs that recognise and work with non-operating women land owners, both resident and absentee are becoming increasingly crucial to conservation efforts. 'The number of these non-operating landowners is growing – in some regions of the US the amount of farmland rented for farming is nearing 50%... we are seeing more and more women landowners, as well as more and more landowners who are several generations removed from the farm,' Professor Petrzelka notes. Conservation efforts in the farmlands of the Great Lakes Basin watershed have been of particular interest in recent years, due to massive toxic algae blooms in the Great Lakes caused by fertiliser runoff from local agriculture. The Great Lakes hold the vast majority of the surface freshwater in the United States, and provide drinking water for millions of Americans. Keeping this water clean and safe is a conservation priority.

Professor Petrzelka and her colleagues are working with local agricultural supply retailers, non-operating land owners, and farmers in Great Lakes watersheds in New York and Ohio to increase conservation knowledge and improve communication between these groups. Educational programs enlighten owners and farmers on the importance of soil health to both their crops and the larger community, while structure is provided to help owners form longer term leases with renters that incentivise conservation efforts and help alleviate some of the financial risk to the renter for starting conservation efforts.

Empowering Female Land Owners Worldwide

Professor Petrzelka's work at Utah State University and with AFT helps to empower non-operating land owners to take leadership positions in the management of their property and impact their communities in positive ways. Their work in the Great Lakes Basin will improve the environmental health of the watershed and be of benefit to Americans. But she hopes to take everything she learns from the three-year project to continue building and refining agricultural conservation programs worldwide. 'I have begun working with colleagues in the Czech Republic, which is experiencing a high rate of non-operator landownership of their agricultural land,' she explains. 'Our hope is to share experiences from our research and our applied work in the field and illustrate that non-operator landownership is not just a US issue, but a European one as well.'

Meet the researcher

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Professor Peggy Petrzelka completed her BA in Political Science from College of St. Thomas, St. Paul, in 1984, before pursuing graduate work at Iowa State University, completing an MS in Rural Sociology in 1991 and defending her PhD in Sociology in 1999. She joined Utah State University in 2001, where she currently serves as a Professor of Sociology in the College of Humanities and Social Sciences. In 2013, she began partnering with American Farmland Trust to harness the results of her research. Her research focuses on how social dynamics such as power and gender play out on the land, with current projects examining the role of female land owners in the farmlands of the US Midwest, the social and environmental implications of varying forms of land tenure, and the social impacts of hydraulic fracturing in the US.

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American Farmland Trust
SAVING THE LAND THAT SUSTAINS US

College of Humanities and Social Sciences
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STRATEGIES FOR SUSTAINABLE FOREST MANAGEMENT

Forested land improves urban water quality, but needs to be appropriately managed and protected from the impacts of land use changes. **Professor René Germain** at SUNY ESF is passionate about improving the sustainability of forest management, and carries out research and outreach programs to build knowledge and explore better management practices.

The Model Forests of the New York City Watershed

Forests are vital habitats for many species and make important contributions to human welfare. As well as timber, they provide essential ecosystem services such as absorbing carbon dioxide, moderating weather, and purifying air and water supplies for both rural and urban communities. Despite this, forested land is threatened globally due to increasing demand for land as a result of a growing human population. Well-designed management strategies are essential to ensure the long-term future of forests and all the benefits they offer.

Professor René Germain is a scientist working to improve the sustainability of the forest products industry in the US. He explores how land use changes and different forestry systems affect management practices. Based at the State University of New York College of Environmental Science & Forestry (SUNY-ESF) in Syracuse, New York, he also has first-hand experience of forestry through previous work as a forester and vice-president of a lumber company. This experience has given him particular insights into what makes a sustainable forestry system, and which knowledge gaps need to be filled to improve management.

Professor Germain is convinced that a critical aspect of achieving better forestry practices is targeted outreach and education programmes. Since 1998, he has coordinated the Model Forests Program, a network of four demonstration forests in the New York City (NYC) Watershed that are designed to showcase good forest stewardship. The NYC Watershed is a 2,000 square-mile lushly forested area containing nineteen reservoirs, three controlled lakes, and three major watersheds – the Catskill, Delaware, and Croton.

The Model Forests are collaborative projects. Though overseen by the Forestry Program of the non-profit NYC Watershed Agricultural Council (WAC), multiple organisations contribute to their ownership, maintenance and stewardship, including the US Forest Service, the NYC Department of Environmental Protection, SUNY-ESF, Cornell Cooperative Extension, Frost Valley YMCA, and Green Chimneys. The Model Forests 'outdoor laboratories' are for scientists to compare the long-term effects of different silvicultural approaches on forest ecosystems and water quality. They also model best management practices (BMPs), widely-used tools to maintain water quality and improve the sustainability of forestry operations.

Each Model Forest is linked to an environmental education centre. Visiting these forests is an exciting opportunity for landowners, policy-makers and the public to see first-hand how logging, nature, and water quality protection can coexist. The experience helps visitors build understanding and find common ground, improving their ability to contribute to sustainable and economically viable forestry.

In their 2007 article in the *Journal of Extension*, Professor Germain and his colleagues discuss the effectiveness of the Model Forest approach. 'The Frost Valley

Model Forest is used extensively by the YMCA as part of their environmental science curriculum to 30,000 students annually,' they explained. 'Thus far, the Frost Valley YMCA Model Forest represents our greatest success story in terms of truly integrating research, Extension, and outreach.' On the research front, in a 2011 article in the *Journal of Forest Ecology and Management*, Professor Germain and colleagues from the US Geological Survey report the relation of harvesting intensity to changes in soil and stream chemistry, confirming that light forest thinning operations have negligible effects on water quality.

Forest Fragmentation Can Accelerate Development

One of the greatest threats to water quality in the NYC Watershed is the loss of forestland through land use change. One such change is the gradual transition away from forest resource management to rural residential use through the fragmentation of forests into small 'parcels' of land, which are then used for development.

The increasing parcelization of forests has strong implications for resource management. When parcels drop below a certain size threshold, managing them becomes economically unviable, further increasing the incentive for owners to allow development. This also means that managers are less likely to be able to implement better forestry practices due to their higher costs.

In NYC, parcelization could have a significant impact on the city's water quality and come with a heavy price-tag for the taxpayer. The NYC Watershed supplies over 9 million people in the greater NYC area. Because the Watershed's forests are so efficient at filtering the water supply naturally, the US Environmental Protection Agency has granted NYC a waiver from the federal requirement that surface drinking water supplies pass through a filtration plant. By not needing to build this plant, NYC saves an estimated $10 billion in construction costs and more than $300 million annually in operating expenses.

WAC, NYC and New York State are all keenly aware of the importance of the Watershed's natural filtration, and together they have protected 34% of the Watershed's land area through fee purchases and conservation easements. However, the remaining 66% of Watershed land is privately owned and vulnerable to land use change.

Although there is anecdotal evidence of increasing forest parcelization in the NYC Watershed and beyond, there has been little systematic assessment of its impacts. One open question has been the exact rate at which parcelization is occurring. Professor Germain and his colleagues explored the average parcel size of forests in the NYC Watershed over the 16-year period between 1984 and 2000. The results, published in the *Journal of Forestry* in 2005, show a significant decline in parcel size from 19 to 16 acres. A 2016 paper published in the Journal of Conservation Planning reports an average parcel size of 13 acres in 2010, with one-third of the parcels below the resource management acreage threshold of 30 acres, as reported by Professor Germain and his colleagues in 2009 in the *Journal of Forest Policy and Economics*. The general picture is of increasingly fragmented, small forest parcels, with a continued shift towards the lower size categories. This is worrying news

for the stability of NYC Watershed forests and their ability to filter the water supply, as these smaller forest parcels are more vulnerable to development.

Residential land is associated with impervious surfaces such as brick, stone and pavement, which have a much lower ability than forested land to absorb water. Increasing impervious surface area can raise the likelihood of flooding and of transporting chemical residues into the water supply. Although denser settlements generally have a higher level of impervious surfaces, even low-density rural residential development can damage water quality, through runoff that transports pollution from lawn chemicals, septic systems, animal waste and sediment. Recent research has shown that water quality can be harmed when impervious surfaces occupy as little as 2.4% of a watershed's area.

In their 2012 article in the *Northern Journal of Applied Forestry*, Professor Germain and his colleagues used a combination of field data, digital imagery and landowner surveys to compare the land cover of NYC Watershed parcels subdivided between 1984 and 2005 to that in parcels which remained intact. They showed that with each new subdivided parcel, impervious surface area increases by an average of 3200 square feet (297 square meters), which is likely to impact local water quality. Improved management practices have been effective at reducing pollution from agriculture and forestry operations, and Professor Germain and his team believe that new policies could continue this positive trend, by regulating residential development.

Towards Better Management Practices

BMPs are an essential way of improving forest management. Properly implemented, they can protect local water from the soil erosion and sediment transport that often accompany logging, which could otherwise pollute the water supply. From 1997 to 2015, the non-profit WAC promoted BMPs in the NYC Watershed by funding private landowners to get forest management plans. The plans included detailed information about ways these landowners could manage their forests. Professor Germain led a formative evaluation of BMP implementation in the early 2000s, reporting in a 2005 article in the Northern Journal of Applied Forestry that despite this extensive outreach and extension effort by WAC to promote BMPs, implementation was unacceptably low.

In 2009 and 2011, WAC worked with Professor Germain and his colleagues to find out whether these plans really led to better practices on the ground. They conducted field surveys of recently logged private forests in the NYC Watershed and scored them on their use of BMPs. The results, published in the *Journal of Forestry* in 2013, showed that forests with management plans only scored better in two of six BMP categories.

Because of these research findings, WAC changed its approach to landowner outreach. The non-profit redirected its management plan funding to help landowners enroll in New York's Forest Tax Law Program, which lowers a landowner's property taxes if the owner agrees to follow their management plan and not parcelize or develop their land for ten years. WAC also created a new website for landowners, MyWoodlot.com, that provides owners with ideas and projects to promote forest stewardship.

In addition to working with landowners, WAC also promotes BMPs with foresters and loggers. BMPs often come at a high cost to loggers, so WAC's BMP Program pays loggers a cost-share to install BMPs on forest roads, skid trails, and stream crossings in the NYC Watershed.

Although cost-share programs like WAC's can be an important mechanism to improve forest management practices, their role in ensuring a stable future for the forest depends on whether loggers are able to install BMPs efficiently. If the cost of implementing BMPs is prohibitive, loggers may not use them even if offered a cost-share.

Professor Germain and his collaborators used a case study and a survey to assess how BMPs affect logging costs and productivity. The results, published in a 2017 article in the *Journal of Forestry*, show that both of these effects are highly variable, with BMP costs ranging from $0–62 per acre, and productivity decreasing by 0–20%. Professor Germain's team found that certain operation strategies can reduce these losses, such as using a dozer rather than a grapple skidder to install water bars, and installing BMPs with machines otherwise not in use. Professor Germain and his team also call for a fairer distribution of BMP costs, with other stakeholder groups like sawmills and landowners providing some of the funding instead of relying on loggers alone to absorb the loss.

Ensuring the sustainability of forestry is critical for the viability of the forest products industry. In turn, a viable forest products industry provides income that helps landowners keep their forests as forests, rather than parcelizing them into rural residential developments. Thanks to the efforts of Professor Germain and his colleagues, our improved understanding of land use change drivers and BMPs will help secure forests and their roles in natural and human wellbeing for future generations.

Meet the researcher

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Professor René Germain obtained his BSc in Forestry from the University of Vermont in 1983. In 1988, he received his Master of Science in Business Administration from Boston University, and went on to complete a PhD in Forest Resources Management in 1997 at the SUNY College of Environmental Science & Forestry. He continued his work at SUNY ESF, as Assistant Professor from 1998 to 2003, Associate Professor from 2003 to 2010, and Professor from 2010 onwards. He has also worked in the forest products industry. Professor Germain focuses on research into sustainable forest systems and outreach through the NYC Watershed Model Forests Program, which he coordinates.

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USING MOTHER NATURE TO HELP CLEAN UP MOTHER NATURE

Mycologist and Environmental Engineer **Dr Irina Druzhinina** and her colleagues at the Biochemical Technology Research Division of the Institute of Chemical, Environmental and Biological Engineering, TU Wien in Vienna, Austria, are looking for a compound to use in the disposal of plastic waste material to prevent environmental pollution. They think this substance may have already been invented – by a naturally occurring fungus.

Dealing with the Unnatural Naturally

'Plastics' – that word was one of the top 100 movie quotes of all time, from the classic film The Graduate. The advice given to graduating Benjamin Braddock, played by young Dustin Hoffman, was that there was a great future in plastics. And after World War II, this was certainly correct. The technological explosion of knowledge in the petrochemical industry has had a profound effect on our lives. You're probably wearing clothing made out of polyester, such as Terylene or Dacron. You may be drinking bottled water or soda from a plastic bottle. And you may live in a building with insulation or other components made of Mylar. All of these substances are forms of polyethylene terephthalate, or PET – polymerised units of the monomer ethylene terephthalate. This ubiquitous material is the fourth most common plastic in the world today, following polyethylene, polypropylene and polyvinyl chloride. And as just about every news article on environmental pollution will tell you, such refuse as discarded plastic bottles are the bane of conservationists the world over. What are we to do with all that discarded plastic? It may be that Mother Nature already has that figured out, at least to some extent.

Although PET is so familiar to us, in an evolutionary sense it is a newcomer on our planet. The natural mechanisms for its recycling may evolve in future, like what has happened for cellulose, for example, but this process is very slow and takes millions of years. For now, PET is not easily biodegradable and we, humans, are in charge for its recycling. However, there are naturally occurring polyesters that can be degraded by microorganisms. The plant cuticle – the hydrocarbon, lipid and wax film that covers the outer surfaces of leaves to protect it from external invasion and to prevent water loss by evaporation – is a type of polyester, generically called *cutin*. And as you might expect, there are naturally occurring enzymes that can break down cutin. Some species of fungi and bacteria make cutinases that are able to hydrolyse cutin, so that the fungus can invade the plant's leaves and basically enjoy a good salad. After all, microbes have to eat, too. Of course, cutin is not a plastic. But the mechanism to degrade polyesters clearly exists in nature – it's just for scientists to find a way to make it applicable to artificial polyesters like PET. Dr Druzhinina and her colleagues do extensive research on that very question.

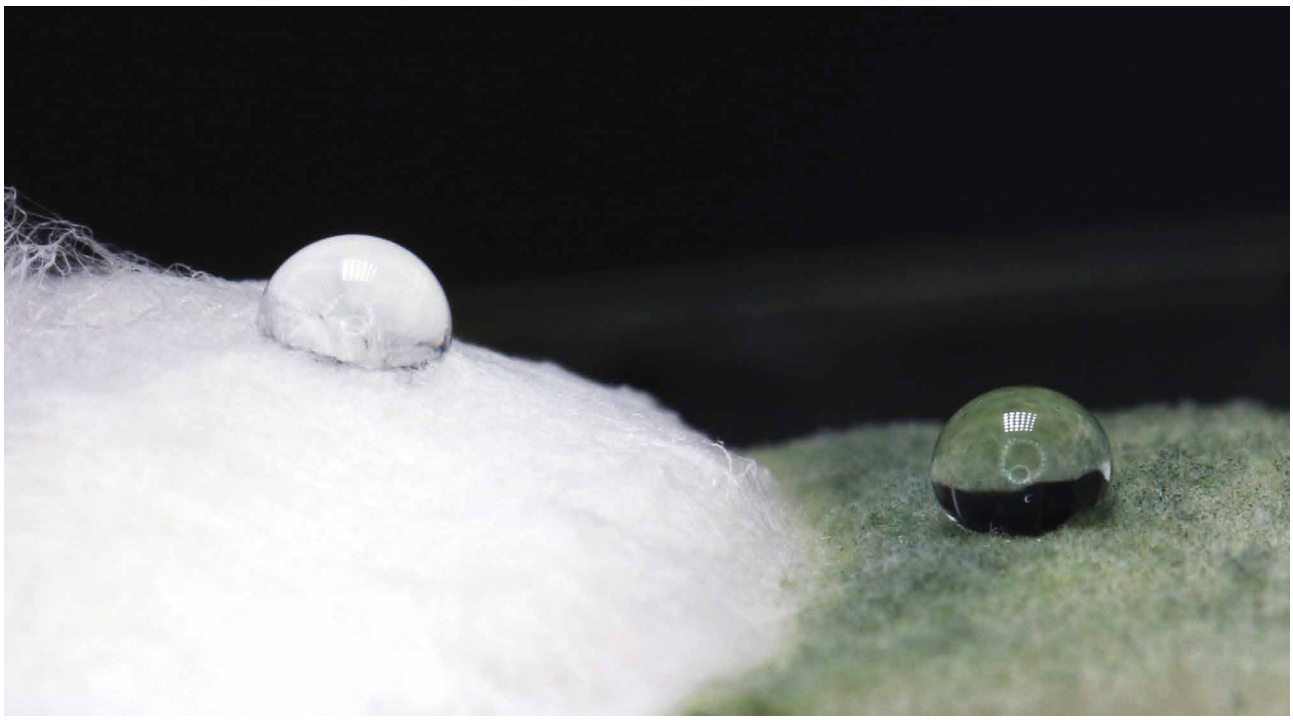
Knowing a Fungus Inside and Out

Dr Druzhinina has studied a particular fungus for over 15 years – the genus *Trichoderma*. *Trichoderma* is employed in industry to produce cellulolytic enzymes. Various species of this fungus are known as environmental opportunists, as they are common in a diversity of habitats such as soil, other fungi, dead wood and living plants, the world over. *Trichoderma* is also used as a biofungicide and biofertiliser for plant protection in organic agriculture, being widely considered as a plant-beneficial fungus.

Since these fungi are known to be extremely industrially important, they have been studied extensively. Therefore, much is known about their physiology, genetics and the various enzymes and chemicals they produce. Dr Druzhinina herself has published a number of papers dealing with the evolution, physiology, ecology and genetics of *Trichoderma*. In fact, in 2011 Dr Druzhinina and colleagues published an article in the Nature Reviews Microbiology that clarified the ecological genomics of *Trichoderma* fungi by integrating the knowledge on the genomes of some species with physiological and ecological characteristics of a wide array of species. Clearly, Dr Druzhinina has become quite an expert on the fungus *Trichoderma*. This put her in an excellent position to see a possible extension for *Trichoderma* to other branches of biotechnology, such as the plastics pollution problem.

There's Degradation and Then There's Degradation

Getting rid of plastics is not an easy thing to do. Ideally, enzymatic recycling of polymers and especially of PET would separate the



polymer into its constituent pieces, namely ethylene glycol and terephthalic acid. These compounds are pretty valuable and can be reused in a variety of chemical processes, including the production of more PET. Currently, however, PET recycling either requires pure PET fractions – which is difficult practically, because of all the leftover contaminants we throw away with our bottles and other trash – or you have to deal with the contaminants, which complicates the process. Also, one reason PET is so useful in bottles and other manufactured materials is that it repels water, or is hydrophobic. In order to recycle it, the PET has to be modified to increase its hydrophilicity – its 'wettability'. Synthetic polymers such as PET also show excellent chemical resistance, but this feature makes them very difficult to mix with various chemicals to degrade them, and poses a big hurdle in the degradation process. Currently you need strong treatments, such as the use of harsh chemicals like concentrated acid or alkali or different types of plasma furnaces, to add reactive chemical groups to the PET molecules to cause degradation. It is no surprise, then, that enzymatic degradation has recently received considerable attention. Using enzymes to degrade PET is presumably more environmentally friendly than current techniques. No harsh acids or alkalis, not to mention hot plasma furnaces, are needed if you use enzymes that work near room temperature.

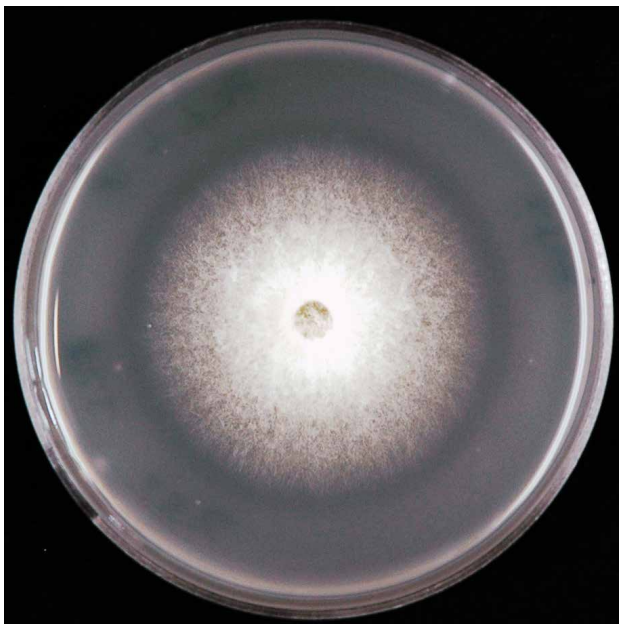
A number of different enzymes are already known that can degrade PET to some extent. Among them, cutinases seem to be the most effective. There is one problem, however – the process is slow. Apparently PET is so hydrophobic that some enzymes have trouble 'sticking' to the PET to get the job done. Regular plant cutin isn't so tough, but PET was designed this way. Thankfully, however, scientists have described another type of substance that can help here – small secreted cysteine-rich proteins called hydrophobins. These are unique proteins produced by fungi. Threads of fungi (called filaments or hyphae) develop either on the substrate that the fungus is eating or directly in it. This means that the good attachment is a critical function for the survival of the fungus. Here hydrophobins help: they are amphiphilic and naturally adhere to both hydrophobic and hydrophilic surfaces changing their property towards the opposite. In other words, hydrophobins can stick a fungus or an enzyme practically to any surface including the hydrophobic PET, allowing the enzyme to do the work of degrading the PET.

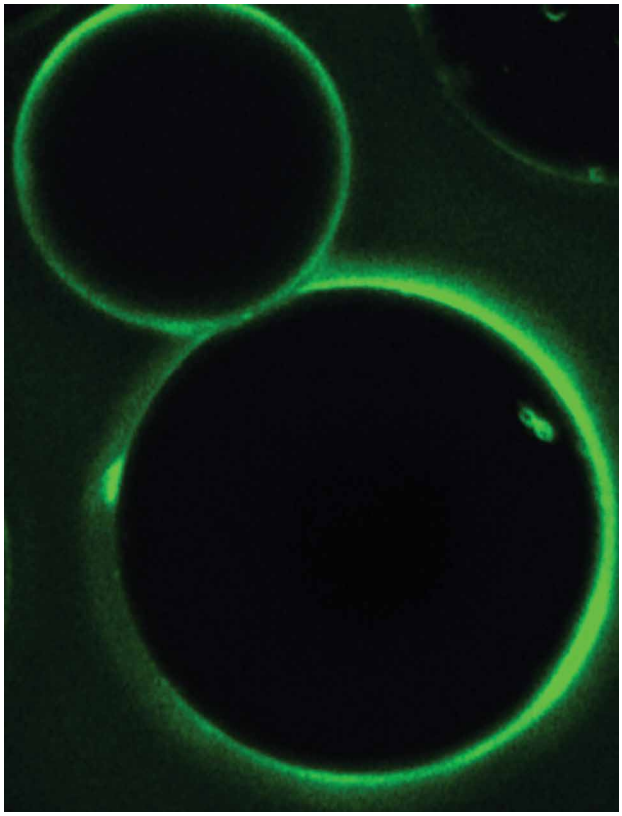
This is where Dr Druzhinina is applying her intimate knowledge of *Trichoderma*. Interestingly, species of *Trichoderma* that are particularly opportunistic happen to have an unusually high number of genes that code for the hydrophobins. Moreover, each species seems to have its unique set of these proteins that for several hundred *Trichoderma* species creates a treasury of surface-active proteins with potentially useful properties.

'This is a unique property of *Trichoderma*,' Dr Druzhinina tells us. 'We already know the functions of some hydrophobins but we also need to understand why so many of them are needed for the daily life of the fungus.' She and her colleagues had already characterised several novel hydrophobins and also revealed evolutionary mechanisms that have resulted in the outstanding diversity of hydrophobin-encoding genes in *Trichoderma* species. She knew all about *Trichoderma* hydrophobins and this challenge of looking for their ability to change properties of hydrophobic surfaces including plastics was right up her alley.

Not One Magic Bullet, but Many!

Dr Druzhinina and her co-investigators started with the idea that hydrophobins could possibly be used to modify PET surface in order to make it more accessible for enzymes. They looked at a diversity of *Trichoderma* species for possible hydrophobins and found several types that nobody had described before. These hydrophobins proved to be extremely difficult to handle, due to them being very surface active, or 'sticky', and small. Therefore, the team then spent several years developing systems to produce these hydrophobins in both bacteria and fungi – in what the biotechnologists refer to as their 'cell factories'. 'Agnes Przylucka, who researched hydrophobin production in the course of her PhD thesis, found that the

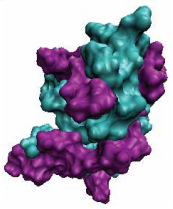




properties of hydrophobins made in different systems – in yeasts, in moulds or in bacteria – are essentially different. All systems allow us to obtain active proteins but their good and bad properties – both are based on amphiphilicity – vary depending on the platform,' Dr Druzhinina explains. The team developed a pipeline for the characterisation of hydrophobin properties based on their physical-chemical methods and computer modelling.

In addition to investigating natural diversity of hydrophobins, Dr Gunseli Bayram Akcapinar, who spent her two-years sabbatical in TU Wien working on hydrophobins, inspired the team to establish the 'rational design of hydrophobins', i.e. to engineer new versions of these compounds in the lab. 'We looked at hydrophobins in evolutionary perspective, modelled their structure, and then, based on this knowledge, we decided which amino acids should be replaced in order to get more surface active hydrophobins,' Dr Bayram Ackapinar explains. 'With this approach we may achieve a kind of fine tuning of a particular hydrophobin for a particular polymer/enzyme combination.' These rationally designed hydrophobins were then also produced in the team's cell factories and tested for the ability to interact with an array of synthetic polymers including Mylar, Capton and PET.

To allow the team visualise how hydrophobins bind to surfaces, other PhD students of Dr Druzhinina, Feng Cai and Tatyana Yemelyanova, engineered fluorescent hydrophobins in order to see how they behave under natural conditions such as on plant roots or other fungi, and on glass surface and synthetic polymers.



From their research, Dr Druzhinina and her colleagues can see that although some hydrophobins can aid biodegradation of PET, the interaction between them and various *Trichoderma* enzymes is a way more complex than initially thought. Interestingly, the understanding of the physical and chemical properties of individual hydrophobins helps to predict their function in the fungus, i.e. in the natural habitat. In turn, when the function of the protein is known, the search for the proteins with specific desired function becomes more rational. This means the team continues their work by expanding their knowledge on *Trichoderma* hydrophobins and on ecological genomics of this fungus and on other fungi in its dwelling.

Perspective: Searching for the Right Microbes in the Right Place

Hydrophobins, however, do not perform the degradation, but only aid the enzyme. The team has an idea that the leaves of gigantic tropical trees with thick cutin layers is the right place to search for microorganisms that are capable of degrading plastics. The phyllosphere, or aerial parts of plants, is an ecosystem that hosts a large and diverse microbial community. 'The adaptation of these microorganisms to their habitat requires the development of specialised functions such as efficient attachment to the leaf surface, resistance to oxidative stress and survival in an oligotrophic environment,' explains Dr Marica Grujic, a biochemist working in Dr Druzhinina's lab. To solve these challenges, phyllosphere microorganisms produce enzymes that hydrolyse not only lignocellulose (a major component of plant matter) but also polyester polymers such as cutin. As modern synthetic polymers such as PET and many others have structural resemblance to cutin, it can be assumed that phyllosphere microorganisms may be also capable of degrading plastic waste. Microbiologist Mohammad Rahimi has already isolated several hundred of microorganisms from phyllosphere. Some of these fungi are promising for plastic degradation. 'We hope to find both: novel hydrophobins or novel enzymes such as cutinases or other enzymes that may degrade not only plant biomass but also synthetic polymers,' Dr Druzhinina explains.

By this integrative approach, they also hope to find other types of hydrophobins or similar proteins that might be useful for industrial applications, where modification of surface properties is important: creating emulsions, coating plastic nanoparticles for drug delivery, and of course, degrading PET. That way instead of fouling up the scenery and polluting the rivers, they can be broken down and recycled to make more bottles and clothing for all of us.

Meet the researcher

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Dr Irina Druzhinina received her background education in mycology in Moscow State University in Russia and pursued her doctoral studies at the Institute of Botany, University of Vienna, where she was awarded her Doctorate of Natural Sciences in 2001. After her training, Dr Druzhinina joined the faculty of the Institute of Chemical, Environmental and Biological Engineering at TU Wien, Vienna, where she is currently Associate Professor leading the Microbiology group in the research division of Biochemical Technology.

Dr Druzhinina's research interests include applied microbiology and biotechnology, comparative genomics and molecular evolution. She has authored or co-authored over 150 articles published in peer-reviewed journals and other professional proceedings. She lectures in and teaches microbiology, genomics, bioinformatics and molecular evolution. She also has developed online courses in protein phylogeny and an on-line laboratory on applied bioinformatics.

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Scientific papers of Druzhinina's group on hydrophobins may be found in NCBI PUBMED
https://www.ncbi.nlm.nih.gov/pubmed/?term=druzhinina%20hydrophobins

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CREDIT: Dr Champagne's research group

WASTEWATER IN HIGH LATITUDES

Dr Pascale Champagne and her environmental engineering group at Queen's University are taking a close look at wastewater treatment ponds across Canada, where climate zones range from temperate to high-arctic. By investigating the role of algae and microbes in these ponds and how they respond to temperature and sunlight, Dr Champagne hopes they can eco-engineer a modern model for treating wastewater in Canadian climates.

The Problem up North

In an effort to reduce the 150 billion litres of effluent released into the environment every year, Canada is adopting tough measures. In 2012, the agency Environment and Climate Change Canada passed strict regulations that mean many operators will need to improve their current systems to treat wastewater. Some have estimated that these upgrades could require a staggering investment upwards of $20 billion.

Small municipalities may bear the brunt of these costly upgrades. More than 3,500 wastewater treatment facilities are in operation throughout Canada today. Many of these use wastewater treatment ponds or stabilisation ponds, that are relatively low cost and useful for small, remote or rural towns in Canada. In the province of Ontario alone, there are 137 such lagoon systems.

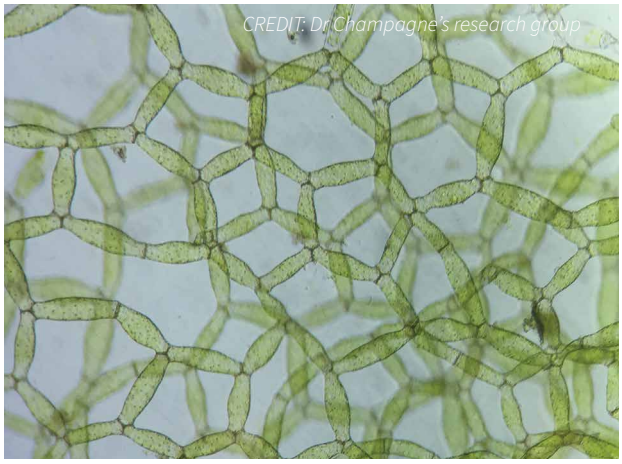
Typically, wastewater treatment ponds work by first reducing the amount of total solids, which settle out and are processed by anaerobic bacteria in the sediment at the bottom of the pond. After the solids load is reduced, algae, aerobic microbes and sunlight kick in to remove organics, nutrients and pollutants, as well as disinfect the water. Until now, little research has been done to understand how these ponds work in extreme climates like those found in higher latitudes.

One thing seems certain at higher latitudes: the performance of wastewater ponds can become unreliable depending on the time of year. In the summer months, long days with increased sunlight and temperatures can cause algae growth to explode, leading to detrimental water quality in the ponds. High algae growth can cause pH levels to rise and can reduce the amount of sunlight and ultraviolet radiation necessary to disinfect water. However, the high pH levels can also contribute to disinfection.

On the other hand, wintertime at higher latitudes brings much shorter days and colder temperatures than those experienced in temperate zones. Freezing causes any biological activity in the ponds to slow or cease, and algae hardly grows at all. This means that wastewater treatment ponds effectively stop working for several months of the year. However, the extended photoperiods at higher latitudes can also contribute 24 hours of treatment in algal ponds, which could be beneficial.

The complexity of these systems can lead to confounding results and potentially affect the effluent from these systems, which can detrimentally impact aquatic environments downstream. Hence, a clearer understanding of the synergistic responses in these systems is critically needed. Understanding how waste stabilisation ponds work in both summer and winter at higher latitudes is a chief aim of Dr Champagne's research team, especially in light of the 2012 wastewater regulations. Her team's ongoing projects in Loyalist Township, located in temperate Ontario, and in the territory of Nunavut, located in the far Arctic north, make for appropriate case studies.

‘The results we have found thus far have been very promising in terms of making wastewater treatment and the release of treatment effluents to receiving environment more achievable particularly in small, remote and rural communities, as well as developing countries.’


CREDIT: Dr Champagne’s research group


CREDIT: Dr Champagne’s research group

The Loyalist Project – Wastewater in a Temperate Zone

The Township of Loyalist is located in a temperate climate zone in central eastern Ontario near Lake Ontario and Canada’s southern international border. There, the Amherstview Wastewater Treatment Plant uses effluent polishing and storage lagoons to treat their effluent before it is discharged back into the environment. Eventually, Amherstview effluent drains into Lake Ontario, which is a source of drinking water for many municipalities.

Like many of its counterpart lagoon systems, algal blooms grow in the Amherstview ponds during times of the year when temperatures and sunlight increase. The conditions caused by excessive algae growth can lead to high pH events that can exceed acceptable and regulatory levels.

To tackle the problem, the Loyalist Township’s Utilities Division has partnered with Dr Champagne’s research group at Queen’s University to improve upon their existing wastewater treatment facility. They’ve decided to incorporate an engineered constructed wetland, one of the planet’s most effective filters, into Amherstview’s facility. Wetlands, the so-called ‘kidneys of the natural world’, are known to be effective in controlling storm water flow, as well as reducing pollutants in effluent discharges. ‘This will be an exciting opportunity for our group to research, since the issues being addressed are very important to small, remote and rural communities across Canada,’ Dr Champagne tells us.

Her team proposed that they convert one of three effluent polishing and storage lagoons in Amherstview’s treatment system to an engineered constructed wetland. The idea was that by adding a manmade wetland to the final stage of wastewater treatment they could effectively attenuate, or ‘polish’, the effluent being returned to the environment.

Before eco-engineering a wetland system, however, the researchers needed to characterise existing conditions at Amherstview. They also had to investigate the feasibility and effectiveness of wetlands to treat its elevated pH levels along with any other water quality issues that could inadvertently arise. So for three years they conducted several preliminary studies that included collecting chemical, physical and biological data on the existing effluent polishing and storage lagoons that were experiencing high algae growth. They also investigated the interactions of these parameters to help identify the conditions that create excessive algae growth and high pH. Peat and topsoil were identified as the two best substrates to allow plant growth and effective pH attenuation from the discharge at Amherstview.

Using these preliminary results, the Queen’s University-Loyalist partnership eco-engineered a pilot-scale constructed wetland, which was finally constructed in the fall of 2016. The area of the wetland is approximately 25,500 m2 in size and consists of twelve 80 x 25 m cells up to 1 m in depth. As planned, it is located downstream from the two remaining effluent polishing and storage lagoons that are similar in size. Water from these ponds will flow into and over the treatment cells, which harbour native species such as common cattails.

For the next two years, the team will closely monitor performance of the constructed wetland and how well it polishes the effluent coming from the previous ponds. They plan to test different plant species and densities, as well as flow and hydraulic conditions to assess their role in treatment performance and in improving water quality.

An important design feature of their project is that they’ve only selected native species for planting in the wetlands because they are adapted to the unique Canadian climate and are resistant to native diseases. The team also wants to avoid introducing invasive species into the ecosystem.

After the two-year monitoring period, the Amherstview eco-engineered treatment facility will hopefully become an important research and demonstration site for other wastewater treatment scientists and


CREDIT: Dr Champagne's research group

operators. The researchers ultimately want to work towards a treatment system design that reduces high pH and algal load and demonstrates resilience and ease of maintenance.

The project will aid efforts to polish wastewater at existing treatment facilities rather than construct costly new ones. If small wastewater treatment operators could simply add constructed wetlands to achieve desired effluent levels, they could well avoid bearing the brunt of more punishing upgrade costs. Furthermore, the wetlands also could add invaluable habitat for animals, act as wildlife corridors and increase outdoor areas for the public to enjoy.

Tactics in the Arctic – the Nunavut Project

Over 2,000 kilometres to the north of Loyalist and above the 54th parallel, lies Nunavut, a sparsely populated territory of Canada's Far North. In this part of the world, an extremely cold climate and permafrost, along with small and isolated populations have necessarily required that wastewater treatment be kept fairly simple and easy to operate. To treat their wastewater, most of the 25 communities in Nunavut use passive treatment technology such as waste stabilisation ponds.

The region is so far north and cold that that fresh water and wastewater cannot be piped to their destinations because of permafrost, and rather are hauled using trucks. Trucks pick up wastewater from individual homes several times per week and deliver it to a central retention pond system that can hold up to a year in volume of wastewater. The wastewater in the ponds remains frozen and stored for 9 months, from October to June. It is then decanted after melting at the end of the treatment period during the warmer months (July to September).

Not surprisingly due to their remote locations, scientists know little of how well these ponds in the Far North are performing. Pond effluent, however, will eventually be subject to the 2012 regulations for municipal wastewater treatment systems implemented by Environment Canada. Currently, the government has extended a grace period to these communities so that scientists and environmental engineers like Dr Champagne can start to better understand their processes and help improve their performance.

She and her colleagues, Rob Jamieson from Dalhousie University and Geoff Hall from Queen's University, characterised several waste stabilisation ponds in Nunavut from 2011 to 2014. During these four years, they measured water quality parameters such as temperature, pH, conductivity, dissolved oxygen, organics, solids and pathogens. They also sampled and tested wastewater at the beginning and end of the treatment cycle. Among other constraints of the Arctic, they found that the period of time during which algae could grow was short, only about 60 days. If pond conditions were left as they were, they would have to hold wastewater for at least three summer seasons instead of one before effluent was properly treated to regulation levels and released back into the environment.

This study identified the problem as low biological activity and weak algae growth, critical factors in treating wastewater in these Far North ponds. To address this issue, the engineers recommend tweaking the design of current wastewater systems, which often have only one pond. For example, by adding another deeper pond as a first stage, they can remove sediments and allow for better algae growth in a secondary pond. Also, by adding filters known as geotextiles into the system, to remove solids and offer additional biological treatment prior to discharge, they can further treat the effluent before it is released into the environment.

Another tactic in the Arctic is to maximise the disinfecting powers of sunlight in the ponds. In a study conducted by Lei Liu, one of Dr Champagne's graduate students, the researchers also concluded that adding another pond to current single-pond systems would help the process. That way, solids and organic material could settle into a primary deeper pond. In the secondary pond, sunlight could then better penetrate and naturally disinfect the effluent.

Cold climates can diminish the effectiveness of wastewater ponds in treating effluent, but the team's research indicates that they can be modified and adapted to work effectively despite limitations.

What the Future Might Hold

Dr Champagne believes that the team's research could be helpful in other applications, and points to algae as an example. Along with the benefits of using it instead of expensive chemicals in wastewater treatment, algae produced in stabilisation ponds could be utilised by cement companies as a biofuel to produce energy. In turn, these companies produce carbon dioxide, which could be used to promote algal growth in the ponds.

'All of this research interconnects to close the loop making waste products as valuable as possible, and it will hopefully make a lasting positive change on the way waste is currently perceived and employed in society,' explains Dr Champagne. Her team's work will make a positive impact towards global sustainability, and may go a long way in helping communities tackle the issue of treating wastewater in small, remote and rural communities, not only in Canada but also in developing nations.

Meet the researcher

Dr Pascale Champagne

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Dr Pascale Champagne received her PhD in Environmental Engineering in 2001 and MASc in Civil Engineering in 1996, both from Carleton University, Canada. She is currently a Professor at Queen's University in Kingston, Ontario, where she is a member of the Faculty of Engineering and Applied Science Engineering appointed to the Department of Civil Engineering and cross-appointned to the Department of Chemical Engineering. Her research focuses on developing alternate water and waste management technologies, in addition to environmentally sustainable solutions for managing bioresources. In 2014, Dr Champagne was inducted into the Royal Society of Canada's College of New Scholars, Artists and Scientists, a program that recognises emerging Canadian intellects and scholars. Prior to that in 2012, she received the Canada Research Chair Tier II award in Bioresources Engineering at Queen's University. Dr Champagne has authored or co-authored more than 100 peer-reviewed journal articles as well as numerous chapters in books related to bioengineering and biotechnology. She oversees the research of several graduate students and serves on the Water Research Centre at Queen's University.

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GREENING OUR AGRICULTURE

Our previous section has highlighted the huge importance of protecting and restoring Earth's biodiversity, introducing innovative ways to promote and facilitate conservation. To ensure a sustainable future for our planet, the researchers featured in our conservation section employ several innovative approaches, from developing new forest management practices and engaging land owners to reintroducing the near-extinct white abalone back into the wild.

However, with the global human population growing out of control, efforts to sustain and restore biodiversity will inevitably become much harder in years to come. Although it took almost 200,000 years of human history for our global population to reach 1 billion around the year 1800, it took only about a further 200 years to reach 7.5 billion, in April of this year. One of the most significant ways that our growing population threatens biodiversity on Earth is through our indisputable contribution to climate change, which is already negatively impacting many species on Earth. Humans simply taking up space is another huge factor, through urbanisation and agriculture, for example. In fact, according to the Food and Agriculture Organization of the United Nations, an estimated 38.4% of the Earth's land area has already been converted to agricultural land, or a whopping 50% of the 'habitable land' on Earth.

When not practiced in a sustainable fashion, agriculture can often represent the greatest threat to species and their ecosystems, through deforestation, pollution with toxic pesticides, soil erosion, and water contamination, to name just a few.

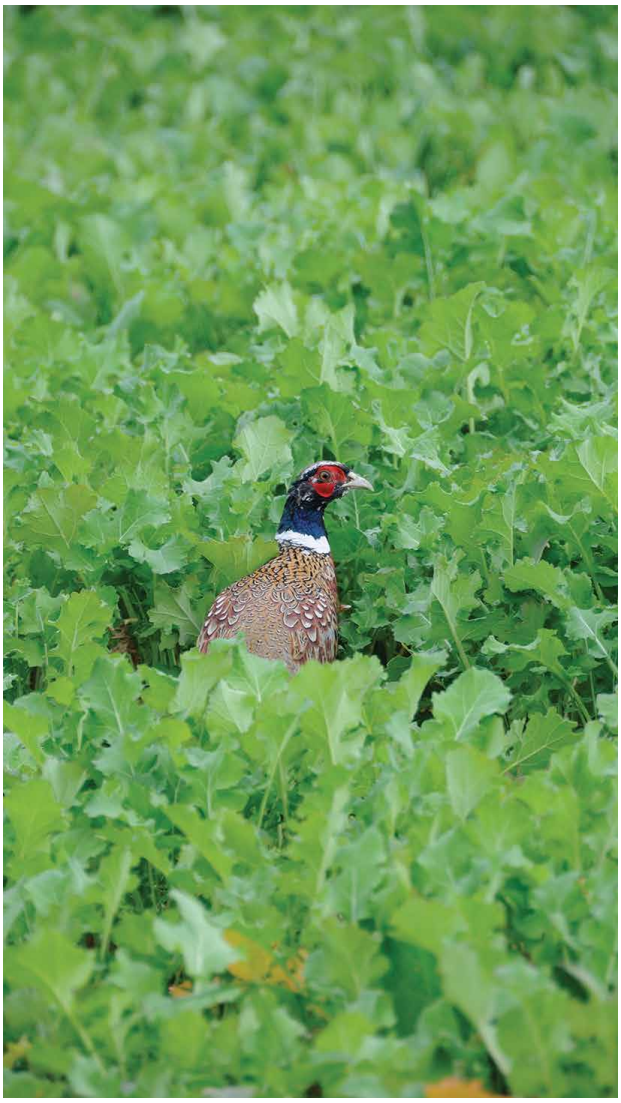
Obviously, in order to feed the ever-growing human population, we need to ensure that our food supply is sufficient. However, in order to ensure the longevity of every species on the planet – including ourselves – fostering sustainable agriculture is equally important. Sustainable agricultural practices can actually help to preserve and restore ecosystems, and actually improve soil health and water quality. Improving our agricultural yields is another important goal, as increased efficiency means a reduced demand for land. Furthermore, climate change threatens our ability to cultivate food, by reducing crop yields through droughts and increasing temperatures. Thus, adapting our agricultural systems to the growing threat of climate change, while minimising agriculture's contributions to climate change, is now more important than ever before.

Improving our agriculture is the theme of the next section of this edition, where we showcase the latest innovations in agricultural science. To open this section, we first introduce an innovative project that revolves around growing crops using aeroponics – a sustainable alternative to traditional crop growing methods. Aeroponic technology involves growing plants without soil, using only a special medium, nutrients and water, and requires only a small fraction of the water and land area used in traditional farming. The project, known as Urban GEMS (Gardening Entrepreneurs Motivating Sustainability) was founded by Dr Deanna Wilkinson of Ohio State University. In addition to providing a means of producing food locally and sustainably, this inner-city initiative gives disadvantaged adolescents an alternative to violence and an opportunity to explore new skills and opportunities, while contributing to the health of their community.

Next, we move on to increasing crop yields by reducing the damage caused by invasive insect pests. Here, we first highlight the work of Dr Mark Hoddle at UC Riverside, who is working to reduce levels of invasive insect species in both cropland and wilderness areas by identifying their natural enemies. By using insects to kill invasive insect pests, Dr Hoddle and his team are eliminating the need for toxic pesticides, which can cause widespread damage to ecosystems.

Also working in the field of pest management is Dr Leonard Coop at the Plant Protection Center at Oregon State University. In the next article of this section, we introduce Dr Coop and his team, who develop tools to predict the arrivals of different pests, and estimate disease infection risks for multiple crops. By providing farmers with pest forecasts, they can then significantly restrict the time they spend spraying their crops, allowing for tighter pest control with reduced use of insecticides and fungicides – helping both farmers and the environment. Also improving agricultural pest control is a team of entomologists at Louisiana State University, who are working to reduce the prevalence of invasive pest species, in an effort to mitigate the damage they cause to rice and other crops. Dr Blake Wilson, Dr Michael Stout and their team apply chemical ecology, induced resistance and integrated pest management strategies as sustainable and environmentally friendly approaches to combatting pests and improving yields.

From rice, we move on to another worldwide staple grain – wheat. Here we feature the research of Dr Guihua Bai and his group at USDA-ARS, who use genomics tools to analyse wheat DNA markers. Their research informs the breeding of new high-quality wheat cultivars, ensuring high grain yields along with disease and insect resistance. Again, this enhanced resistance means that we can reduce our use of toxic pesticides, which devastate ecosystems. Another way to boost a plant's resistance to disease is by ensuring it has access to key micronutrients, which control vital biochemical processes. The next article in this section showcases the work of Dr Wade Elmer and his group at the



Connecticut Agricultural Experiment Station, who are developing clever new techniques to deliver micronutrients to plants. In particular, the team has carried out a study showing that nanoparticles can be used as an effective, practical method to get vital nutrients such as copper into crops. Plants treated with these nanoparticles showed significantly increased resistance to two common fungal pathogens.

And of course, not all of our agriculture revolves around plants – as dairy, eggs and meat also form a significant proportion of our diets. Just like for plants, the meat, egg and dairy industries also suffer significant losses due to disease. Not only do poor health and disease reduce yields and the sustainability of these industries, but because sentient animals are involved, we must also consider the significant welfare impact. Here, we introduce the work of two research teams, each striving to improve the health of poultry, to ensure food security and boost animal welfare.

First up is Dr David Peebles at Mississippi State University, who has dedicated his career to the unique physiological and nutritional needs of meat chickens, and has developed an innovative approach to improve their health and wellbeing, starting in the egg. His team has developed interventions such as vaccines and supplements that can be supplied to chicks before they even hatch, preventing disease and supporting healthy growth and development. Then, our final article in the magazine showcases the work of Dr Chang-Won Lee and his multidisciplinary research team, who partner with farmers, scientists, and governments across the globe to develop ways to understand, predict, and prevent diseases in poultry. The research carried out by Dr Peebles, Dr Lee and their colleagues helps farmers raise happier, healthier flocks, while reducing losses due to disease and development abnormalities.

CREDIT: Doral Chenoweth

GROWING OPPORTUNITY IN THE INNER CITY

Dr Deanna Wilkinson founded the Urban GEMS – Gardening Entrepreneurs Motivating Sustainability – to give disadvantaged adolescents an alternative to violence and an opportunity to explore new skills and opportunities while contributing to the health of their community.

CREDIT: Doral Chenoweth

Planting Seeds of Opportunity

The Urban GEMS (Gardening Entrepreneurs Motivating Sustainability) program, founded by Dr Deanna Wilkinson, grew out of a desire to design a program that would not only keep kids off the streets, but also teach them valuable life skills while providing them with opportunities beyond the neighborhoods in which they grew up. The program is a multifaceted youth development initiative that aims to enrich the educational, personal and career development of young people in high risk communities.

The prevalence of poverty, health disparities, high rates of unemployment, mass incarceration, food insecurity and low educational attainment disproportionately affects urban African American communities compared to their White, Asian and Latino counterparts. Poverty leads to poor health and both of these factors are linked to lower levels of education.

In order to address this vicious cycle, the program cross cuts a number of domains including nutrition, health, wellness, entrepreneurship and community efficacy. Urban GEMS aims to improve health related knowledge, attitudes and behaviours amongst young people, while they gain skills in science, agriculture and food production. The team aims to increase participants' l fresh fruit and vegetable consumption by 50% and improve their knowledge of healthy eating, while learning how to use different systems to grow and harvest crops. However, the effects of a project like this run much deeper than learning how to garden or eat healthier.

The research team want participants to grow in self-efficacy and feelings of belonging. The program encourages teamwork, community engagement and education. Through experimental learning activities, the researchers hope to increase teen engagement in high school. By participating in Urban GEMS, youth are involved in a type of active learning that precipitates high school graduation and prepares them for the workforce and higher education. Participants also gain business acumen through connecting with adults in local food production, and learning how to create a sustainable small business model. Youth participants benefit from the expertise of university faculty, extension educators, community school professionals, volunteers and business sector partners. The first Urban GEMS farm was established in February 2017. Here, youth and staff are growing leafy greens

‘A lot of students are still wrapping their minds around the fact that Ohio State University and people who have resources are willing to invest in them. They are just not used to that. What I want them to know is that they deserve all of the same opportunities as kids who live in communities with more resources.’

CREDIT: Jeff Grabmeier.

on nine towers located in an unused hallway space at an OSU community outreach center. Food grown at the Urban GEMS farm has been donated to help feed the homeless.

As well as developing personally, the young participants are encouraged to become more involved in their community. They are supported in educating others and passing on their newfound knowledge of healthy eating and wellness to their families, friends and other members of the community. Their activities are documented through photos, journals and social media, which can be easily shared and disseminated. As well as engaging the participants in disseminating the project’s activities, this work gives young people the opportunity to learn how to use number and word processing software and be actively involved in the research project. The participants also become familiar with public health campaigns which aim to improve community health outcomes for urban African American men (who are particularly at risk for certain diseases). The plan is that this will increase community involvement in changing patterns of food consumption and inspire young people as leaders and problem solvers in this public health initiative.

Gardening to Engage Youth

So why gardening? At first glance, a garden may seem like an odd choice for an activity aimed at keeping teenagers engaged in school and out of gangs. In some ways, the connection came about by chance, as many great ideas do. Dr Wilkinson first learned about aeroponic gardening from a community workshop and quickly developed a passion for it. Aeroponic gardening is growing plants without soil, using only special medium, nutrients, air, and water. Without the burden of large volumes of soil, aeroponic gardens are particularly suited to non-traditional growing locations and are able to produce wonderful crops of fruits and vegetables in even the most limited of indoor spaces. A good amount of produce can be grown in one narrow aeroponic tower that takes up less than 7 square feet of floor space. The program currently operates at two neighborhoods in Columbus Ohio, selected for high rates of poverty, food insecurity, unemployment, high crime rates, and failing public schools. By 2017, the team was leading youth in growing food in 9 locations with a total of 26 Tower Gardens operating. The primary Urban GEMS implementation sites include a high school and a non-profit youth serving organisation. The research team has plans to expand opportunities for community youth by creating a sustainable business growing food in ‘food deserts’ with as many as 90 gardens in the next five years.

A Blooming Enterprise

Urban GEMS has also brought a new, positive orientation to the team’s research. With the support of the CYFAR grant, Dr Wilkinson and her colleagues collect data from the Urban GEMS program to measure the success of the program and identify areas where it can be strengthened to provide the best benefit for the students. Each site offers a uniform program and curriculum in order to ensure consistency across projects. Students take assessments before entering the program, and continue to be assessed during and after their time as Urban GEMS. These assessments not only look at academic achievement, but also health and wellness measures, and other indicators of improved quality of life for participants. The pre-test data results have been used by the research team to inform decisions about program focus and community needs. Post-test data from the first cohort will be collected shortly

CREDIT: Doral Chenoweth

CREDIT: Big Ten Network

offering a first test of the Urban GEMS model.

The first two years of the project have led to overwhelmingly positive interest from the community with many conversations and activities ignited around healthy eating. The young participants have obtained new knowledge about the many health benefits of the food they grow. The researchers have also observed participants teaching their newfound knowledge to their peers. These students have gained skills as food producers and have had multiple opportunities to prepare, cook and eat their harvests. They have developed the skills to teach others about how the system works from its setup, through its maintenance to the harvest of crops. They have also had the chance to share and trade their fresh produce with local master crop growers.

The team have also observed the participants growing as leaders. Attendance at Urban GEMS activities has steadily improved as young people became more involved in leadership roles. The students have connected with professionals through participation in the Urban GEMS advisory board, led team meetings, interacted with adult advisers and volunteers and gained invaluable experience in public speaking.

The project has also led to productive interactions with the community as a whole. The youth club, which meets once a week to learn about aeroponic food production, nutrition and social justice advocacy, has held successful 'salad in a jar parties', after which they take home food for their families. The young students are also responsible for producing food that is used twice weekly to feed hungry community members who come to the feeding program. Through this, participants learn important life lessons about self-empowerment and becoming a person who gives back to the community.

An Even Brighter Future

It is onwards and upwards for Urban GEMS. The team anticipate that collaboration and financial backing from non-profits and other relevant organisations will help the sustainability of the project. The workforce component was purposefully developed with a view to attracting stakeholders interested in building a prepared workforce for the future.

The Urban GEMS team plan on training middle and high school students in social justice activism around healthy food access in urban areas and conservation of natural resources for sustainable food production. In addition, participants will compare and contrast the aeroponic tower system with traditional gardening in terms of water and energy consumption and the use of climate controlled spaces. The team also hope to secure additional spaces for more towers and explore other ways of generating energy (e.g. using solar panels). Through these endeavors, Dr Wilkinson and her team aim to build a youth development model worthy of replication on a national scale. The program utilises aeroponic food production as a vehicle to enrich the lives and learning of the young people in the program through project based learning around scientific discovery, math lessons, historical analysis, nutritional principles, consumer science education and the creative and language arts. Individual and team based learning integrates literacy, numeracy, teamwork and portfolio production in a culturally relevant, positive and empowering learning environment. There is potential to create a legacy with this approach. Small businesses can develop around vegetable gardening using the aeroponic tower system. Coaching students in various fields exposes them to numerous possible career paths such as agriculture, marketing, public health and other health related areas. The GEMS program model is based on the identified needs of the community and the strengths of assembled partners. By creating partnerships with schools, businesses and communities, the project can help to institutionalise change and create new opportunities within a sustainable project.

The Urban GEMS program is currently operating under a five-year federal grant from the Children, Youth, and Families At Risk (CYFAR) initiative of the US Department of Agriculture's National Institute of Food.

CREDIT: Lindsay Ricart

Meet the researcher

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Dr Deanna Wilkinson began her education with a BA in Sociology from Cornell College in Mt. Vernon, Iowa in 1990, and continued on to receive an MA in Criminal Justice from the University of Illinois at Chicago in 1992, followed by a PhD in Criminal Justice from Rutgers University in Newark, NJ in 1998. She served as an Assistant Professor in Criminal Justice at Temple University in Philadelphia, Pennsylvania from 1998 to 2005, before taking her current role as an Associate Professor in the Department of Human Sciences in the College of Education and Human Ecology at The Ohio State University, Columbus, Ohio. She also holds a courtesy appointment as Associate Professor in the OSU Department of Extension in the College of Food, Agriculture, and Environmental Science. She has dedicated her career to understanding and preventing violence among low-income urban youth, and in 2015 founded the Urban Gardening Entrepreneurs Motivating Sustainability (Urban GEMS) program to provide dynamic educational, career, and community building opportunities to inner-city teens.

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BIOLOGICAL CONTROL: PROTECTING AGRICULTURE AND WILDERNESS AREAS FROM INVASIVE INSECT PESTS

Invasive organisms become pests, in part, because they escape control of their natural enemies that regulate population growth in areas where the invader is native. Biological control attempts to re-associate host specific natural enemies with target pest populations to reduce pest populations to less damaging levels. **Dr Mark Hoddle** of the Department of Entomology and his colleagues at the Center for Invasive Species Research, both at UC Riverside, work on invasive invertebrate pests in California, and develop biological control programs targeting them. This approach can be highly effective, eliminating the need for pesticides, and providing permanent pest control.

Dr Mark Hoddle's interest in insects started at a young age, rearing mantids (*Orthodera novaezealandiae*) in his native New Zealand. 'The subsequent mass production of baby mantids inadvertently lead to me executing my first biocontrol program – releasing these tiny nymphs onto Mum's whitefly infested fuschias!' he tells us. During his undergraduate course lectures in basic insect biology, ecology and taxonomy, as well as Integrated Pest Management, Dr Hoddle built upon this interest and started to consider entomology as a career option. It was then that he realised, 'you could actually get a job doing the things I like doing for fun!' Since 1997, Dr Hoddle has worked as an Extension Specialist in biological control in the Department of Entomology and later as the Director for the Center for Invasive Species Research, both at the University of California, Riverside.

Eradicating an Invasive Palm Weevil

One of the main focuses of Dr Hoddle's work is developing biological controls for high risk invasive agricultural pest species like the glassy-winged sharpshooter (*Homalodisca vitripennis*), and when possible, eradicating incipient pest populations such as highly destructive palm weevils. Palm weevils have long been considered a pest species in Asia, where some species originate. In particular, the red palm weevil (*Rhynchophorus ferrugineus*) has killed millions of Canary Island palms and date palms, and is widely considered to be one of the most damaging insects to palm trees in the world. The closely related *Rhynchophorus vulneratus* from Indonesia, is also a palm killer in its native range.

Measuring between 35 and 40 millimetres, this beetle species has several different colour morphs. One variety is black with a distinctive red stripe on the dorsal side of the thorax, and in 2010, a live male weevil with this colour pattern was found in a dead canary palm in Laguna Beach, California. Dr Hoddle and his colleagues identified this weevil as *R. vulneratus* using DNA analysis, contradicting the previous identification of the weevil as the closely related *R. ferrugineus.*

In California alone, the ornamental palm industry is worth $70 million, while the date industry has an estimated value of $68 million per year. Thus, it was important to act quickly to reduce the spread of this damaging palm pest. Dr Hoddle and his colleagues demonstrated that the species of palm weevil in Laguna Beach was highly attracted to two volatile compounds – odours from damaged palm trees and aggregation pheromones released by male weevils that attract more weevils (male and female) to infested palms. The team placed synthetic versions of aggregation pheromones into buckets with fermenting fruit to attract weevils, which

Gonatocerus ashmeadi. CREDIT: Mike Lewis, Center for Invasive Species Research

The glassy-winged sharpshooter. CREDIT: Mike Lewis, Center for Invasive Species Research

The palm weevil, Rhynchophorus vulneratus, native to Indonesia, was eradicated from Laguna Beach, California. CREDIT: John Kabashima, UCCE

‘The ultimate goal of a successful biological control program is reducing pest populations to less damaging levels, perhaps to the level where pesticides, for example, are no longer needed. This is good for the environment, and for agriculture as it saves the farmer money on pest control.’

Mark Hoddle together with his wife Christina, and Pakistani colleagues from the University of Agriculture Faislabad search for natural enemies of ACP in citrus fields in Pakistan

upon entering the trap weevils fell into an anti-freeze solution and drowned. The use of pheromone traps, together with insecticides and the destruction of weevil-infested palms led to the extermination of palm weevil populations, and successful eradication was declared in January 2015.

Saving our Avocados

Biological control research targeting invasive pest species has wide ranging impacts, and helps to protect a range of our favourite food and drink products, such as avocados, wine grapes, dates, and olives. ‘On average, California gets about nine new arthropod species establishing in the state per year,’ Dr Hoddle tells us. ‘About 33% of these become pests of environmental and/or economic significance.’ Alongside his colleagues, Dr Hoddle has studied the impact of one particular pest of economic importance – the avocado thrips (*Scirtothrips perseae*), which infests 95% of avocado acreage in California. Up to 80% of acreage may require pesticides at certain times; however, as recent evidence continues to confirm the negative environmental impact of commercial pesticides, new biological control methods may be needed to replace them. To do this, Dr Hoddle and his team explored Mexico, Guatemala, Costa Rica, the Caribbean and Brazil, to delineate the native range of the avocado thrips, with the intent of finding its natural enemies. They discovered that the most commonly occurring natural enemies associated with avocado thrips in its home range are predatory species of thrips (*Franklinothrips* spp. and *Aeolothrips* spp.). These wide ranging surveys identified several other species of thrips that have the potential to become severe pest species for avocado if they ever arrived in California.

A novel approach to managing invasive pests is to identify and anticipate potential problems in advance of their arrival, and proactively develop management plans before incursions occur. An obvious target in this regard is the highly destructive avocado seed moth, *Stenoma catenifer.* Multiyear work carried out by Dr Hoddle and his wife Christina in Guatemala resulted in the discovery of its sex pheromone, which turned out to represent a previously unknown class of natural compound. After carrying out these surveys in Guatemala, and later Peru, the team developed comprehensive inventories of natural enemies, predominantly parasitoids (wasps and flies), that parasitise and kill moth larvae and pupae. Identifying both the *Stenoma* sex pheromone and the natural enemies of this notorious pest, in advance of it potentially invading California, is an invaluable insurance policy protecting California’s $275 million per year avocado industry.

Controlling the Sharpshooter

In the 1980s, the glassy-winged sharpshooter was accidently introduced to California from its native range, the south-eastern US, most likely through the import of infested nursery plants. Individual adult sharpshooters ingest 100 times their body weight in fluid from the xylem of plants, whilst also being a vector for the xylem-dwelling bacterium *Xylella*

A Canary Islands date palm killed by R. vulneratus in Laguna Beach. CREDIT: John Kabashima, UCCE

fastidiosa. This bacterium causes several lethal plant diseases, most notably Pierce's disease, which impacts both wine and table grape varieties. Pierce's disease occurs when *X. fastidiosa* blocks the xylem of the plant, preventing water flow and resulting in disease symptoms such as scorched leaves, which can eventually lead to plant death.

Since its initial introduction into California, the glassy-winged sharpshooter population increased, as did plant diseases associated with feeding insects. In 2000 alone, $6.9 million USD was spent on pesticides in an attempt to manage populations, in response to grape losses in San Diego and Riverside Counties due to *X. fastidious* related diseases, which were estimated in excess of $37 million USD. As glassy-winged sharpshooter populations continued to increase in California, the pest was inadvertently exported to Hawaii and French Polynesia through trading live plants. Populations in French Polynesia reached exceptionally high densities, far greater than anything observed in California. Subsequently, the glassy-winged sharpshooter spread from French Polynesia to Easter Island and the Cook Islands. This pest needed to be urgently controlled, and biological control was identified as the strategy most likely to provide significant and environmentally acceptable pest suppression. Natural enemies, in particular parasitoids that attack glassy-winged sharpshooter eggs, were considered excellent candidates for use in a biological control program targeting this pest. Dr Hoddle and his colleagues quickly identified a key candidate – *Gonatocerus ashmeadi.*

'Parasitoids' such as *G. ashmeadi* lay their eggs inside those of the glassy-winged sharpshooter, and the developing larvae kill the sharpshooter egg by feeding on its contents. The team's use of *G. ashmeadi* significantly reduced pest populations throughout invaded areas in California. However, the impact of *G. ashmeadi* has been most spectacular in French Polynesia, where within 7 months of release of this egg parasitoid, glassy-winged sharpshooter populations were reduced by more than 95%. This impressive level of control has resulted in permanently low populations of the glassy-winged sharpshooter in this large island archipelago. A secondary major achievement of the team's highly successful program was the discontinuation of the spread of the glassy-winged sharpshooter throughout the South Pacific. This pest has not been recorded in any additional island nations following the implementation of this biocontrol program in French Polynesia.

Saving Citrus

The Asian citrus psyllid (ACP), *Diaphornia citri,* is a citrus pest with global notoriety because it acts as a vector for a bacterium that causes a lethal and incurable citrus disease called huanglongbing (HLB). ACP-HLB has had a devastating impact on Florida citrus production, and California growers are worried about a similar outcome affecting them, as ACP and HLB are now established in many urban areas in California.

In response to the threat that ACP-HLB poses to commercial citrus in California, a $3 billion (USD) industry, Dr Hoddle and Christina Hoddle undertook a biological control program to target it. To do this, they needed to work for extended periods of time in Punjab Pakistan – part of the native range of ACP. In collaboration with Pakistani colleagues at the University of Agriculture Faislabad, they searched for and identified natural enemies of ACP that could be imported into California under permit for safety evaluations in quarantine. Two parasitoids, *Tamarixia radiata* and *Diaphorencyrtus aligarhensis*, which attack and kill different developmental stages of ACP nymphs were identified and approved for release by federal regulators. One of these parasitoids, *T. radiata*, established readily and spread rapidly by taking advantage of the widespread and dense ACP populations that were infesting urban citrus. This parasitoid, in combination with predatory insects like syrphid fly larvae, have greatly reduced ACP densities, and have probably contributed to its reduced spread in California. However, there is much more work to be done on this problem to prevent a catastrophe similar to that seen in Florida.

Protecting Wildlife

The goldspotted oak borer (*Agrilus auroguttatus*) is a beetle native to southern Arizona, which was first detected in California in 2004. By 2008, it was found to be adversely affecting three species of 'red oak' in the Cleveland National Forest, San Diego. Many infected oaks are large and very old, and as such a keystone species, their loss severely impacts biodiversity. Dr Hoddle and his colleagues collected data on the origin, biology and life history of the goldspotted oak borer, including information on its natural enemies. They also investigated its flight capabilities, including the total distance flown, by using a computerised flight mill. To estimate the distances that insects are capable of flying, entomologists attach insects to the arm of the flight mill, which rotates on a near frictionless spindle. As the insect flies, the arm of the mill rotates on this spindle and a computer records distances flown.

Flight mill data indicated that adult goldspotted oak borers would not have had the ability to cross the Sonoran and Mojave deserts, natural and hostile barriers separating the native range of goldspotted oak borers from the invaded area in southern California. This work suggests that this pest was introduced into the Cleveland National Forest by humans transporting oak firewood from Arizona to California. Human movement of firewood in California has resulted in goldspotted oak borers becoming established in additional areas. This problem is likely to continue as there is no regulation of moving infested oak firewood from contaminated areas to uninfested parts of California. As part of their project, Dr Hoddle and this team identified two species as potential parasitoids of the goldspotted oak borer. Unfortunately, further work suggested that these natural enemies were unlikely to provide adequate biological control of goldspotted oak borers and California's iconic oak forests and savannahs remain threatened by this highly destructive pest.

Meet the researcher

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CLOUDY, WITH A CHANCE OF INSECTS

The Integrated Plant Protection Center at Oregon State University is both a hub for research into agricultural pest management and the host of widely-used pest forecasting tools. **Dr Leonard Coop** and his co-workers plan to improve those tools even further.

Should I plant my crops tomorrow or next week? Answering this apparently simple question is a matter of both vital importance and exceptional difficulty – it underlies the success of our crops (and thus our ability to eat) and yet is almost impossible to answer with any certainty. Indeed, trying to answer this with confidence is a problem which has frustrated humans for millennia.

Early answers were based on simple observations of the natural environment and their correlation with the weather. This was often passed on in the form of sayings – think of 'red sky at morning, sailors take warning', 'clear moon, frost soon', or based on the actions of local animals – low-flying swallows predict rain, cows stand up when it gets warm. These types of predictions tend to be useful in a short term and unreliable way – they help you to plan your farming tomorrow but rarely help you for the next week.

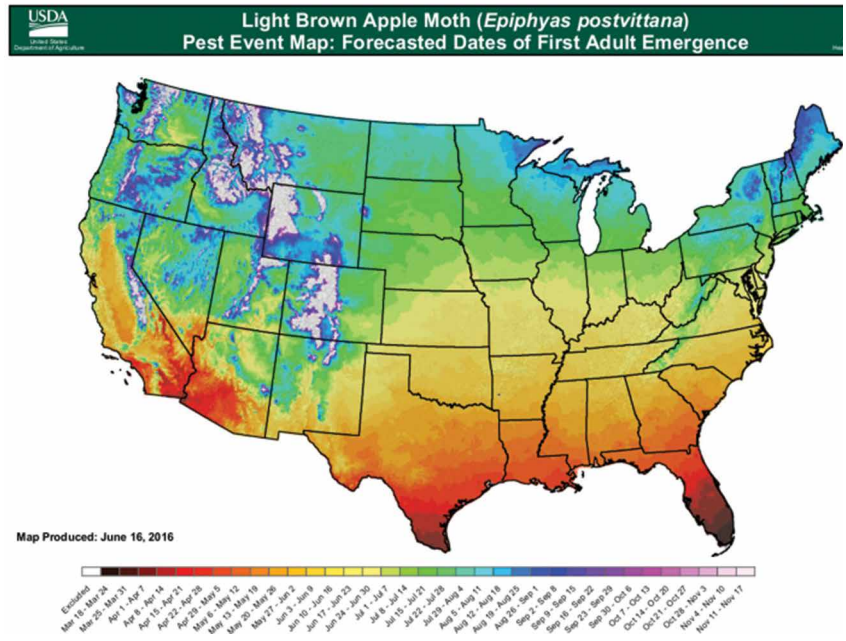
Weather forecasts became more systematised in the 1800s, when the telegraph allowed knowledge of the weather to travel faster than the weather itself. Widespread collection of weather data ensued and allowed rough charts of local conditions to be constructed. This lead quickly to the first published weather prediction in 1861, suggesting fine weather with a fresh westerly wind the next day (the actual weather was cloudy with a southerly wind, beautifully illustrating the difficult relationship that weather forecasters have with their subject matter).

Various advances then led to the notion of numerical forecasting, in which a mathematical model of the atmosphere could be created and used to predict future conditions from the current status. Essentially dividing the atmosphere into a 3D-grid and then modelling the interaction of each section with its neighbours, this method was (to put it lightly) fiendishly complicated. Because of this complexity, useful numerical forecasting only took hold in the 1950s, when computers allowed forecasts to be calculated *before* the weather had come to pass. Steadily advancing computing power and mathematical models have allowed for steadily more accurate models, to the point where 8 day forecasts are usually quite reliable. Getting past this point, however, is where the difficulty starts.

Whether the Weather be Windy

This difficulty arises from the chaotic nature of the weather and atmospheric conditions. Chaotic systems are best described as those in which small differences in initial starting conditions lead to widely differing conditions at later stages. Similar problems apply to weather forecasting – small errors or uncertainties in the initial forecast will rapidly multiply to provide something which is utterly different to the actual conditions. Because of this, longer-range forecasts tend to rely on things such as 30-year normal data – if you know what the temperature has been on June 22nd for the last 30 years, you can be fairly certain that this year's temperature will be around the average of those previous values. This type of prediction is simple to make, often correct, and will *completely* miss every atypical change in conditions. It is, in the jargon of meteorologists, lacking in 'skill'.

Skill is not, it should be noted, the skill of the meteorologist – it instead refers to how well a forecast actually predicts the future. Simply taking a number of previous measurements as your prediction is defined as the skill-less forecast, this is considered to be the



CREDIT: USDA aphis ppt

baseline prediction. Forecasts which predict the weather with better accuracy than this baseline have a higher degree of skill, those with less accuracy can be thought of as having negative skill. The goal of forecast experts is to increase the skill of their models over time.

To improve the overall predictive skill, weather forecasters use a number of ever-more complex mathematical models each utilising increasingly powerful computers. One of the current leaders for longer-term forecasts is known as the North American Multi-Model Ensemble (NMME) – a system that combines models from seven different organisations (including NASA and NOAA) to produce a relatively reliable but low-resolution forecast of conditions out beyond 3 months from today. It does this in part through extensive incorporation of oceanic conditions (including long-term effects such as the El Nino/La Nina oscillation).

Whether You Like it or Not

You may be reading this article and wondering why you should care – tomorrow's temperature is important, but in two weeks? Or three months? For most of us this is not hugely important information. But it is very important indeed for agriculture, because the environment heavily affects both the growth of crops and the growth of the pests that would like to feed on them. Thus, a vast amount of forecasting research is linked to improvements in the current state of Integrated Pest Management.

Integrated pest management, or IPM, can be thought of as the scientific approach to controlling harmful pests. It centres on the idea that you cannot wipe out an entire pest population (at least, not in an economically efficient way) and thus it is better to take action to keep your inevitable pest population below a certain threshold value. This involves a lot of population monitoring and the use of mathematical models to determine when and how much intervention (such as spraying of insecticides) should be performed. Weather forecasting provides a vital part of this approach, in that many serious pest infestations are related to environmental conditions. Let's take two of these in particular – potato late blight and spotted wing Drosophila.

Late potato blight is caused by infection from the rather complicatedly named oomycete *P. infestans*. Infected potatoes will rapidly decay, even in storage, and fields can remain infected for years if some infected tubers are accidentally left in the ground. Blight was one of the major causes of the Irish Great Famine (a natural disaster which led to the death by starvation of about one million people), and to this day the disease causes crop losses worth around $6 billion each year.

The late blight infestation is strongly correlated with environmental conditions, with humidity and temperature playing a vital role in *P. infestans* reproduction and spread. Farmers look for periods of 1–2 days in which the temperature is over 10°C and humidity is over 90% – conditions which are particularly conducive to blight spread – and perform fungicide spraying at these times. Weather forecasting models are able to predict these conditions and so indicate ideal times for treatment – this lies behind a number of current warning systems for farmers such as BLITECAST.

Spotted wing Drosophila, by contrast, are small, fast-breeding insects. They are problematic pests for fruit farmers, laying eggs inside ripening fruit and so causing serious damage to soft fruits such as apricots or berries. Since their first detection in 2008, the flies have been steadily spreading throughout the continental United States. Only a few millimetres long and unable to fly long distances, their rapid spread is mostly assisted by human transport of infected crops. Spotted wing Drosophila tend to emerge from hibernation once temperatures hit 10°C and will quickly search out susceptible fruit to lay their eggs. As such

the ability to predict temperatures at the local orchard level is very important in allowing farmers to plan their insecticide applications.

Prophesying Plagues of Pests

This brings us to the work of researcher Dr Leonard Coop of Oregon State University and his collaborators at Washington State University and Fox Weather LLC. Oregon State University currently hosts USPEST.org, an online portal that allows the general public to connect weather data and forecasts from tens of thousands of weather stations with over 120 models that predict the occurrence of pest arrivals, emergences, peak flights, and disease infection risks for multiple cropping systems. By combining this data, it is possible for farmers to easily view, say, the most likely emergence dates for spotted wing Drosophila, and they can then use this information to plan their insecticide treatment. In particular, recent improvements to the site (including incorporation of Google Maps information) now makes it relatively easy for the public to choose an area of interest, run several types of computer models, and then plot predicted risks on a map of the local area or in interactive graphs over time. This online portal is heavily used, yet suffers from the same difficulties as all weather forecasting – predicting temperatures beyond about 10 days out rapidly becomes very unreliable and so everyone needs to fall back on 30-year-normal data.

To move beyond this difficulty, Dr Coop and his collaborators are adding data developed by other meteorological departments, in particular the long-range NOAA NMME models. NMME models are able to give state-of-the-science estimates for dates relatively far into the future, but these models (and the departments that host them) have a stronger focus on both large-scale weather systems and wide-ranging oceanic conditions. As such, the data they produce has yet to be applied to more specialised and 'small-scale' work such as the forecasting of insect or blight outbreaks.

To fill this gap, the group has blended high-resolution historical data (the 30-year-normal data collected over the decades), lower-resolution but longer-term forecasts (such as those provided by NOAA or in NMME models), and the high-resolution but short-term data available at weather stations around the continental United States. This smoothing process allows each model to fill the gaps left by the other – long-range forecasting can be blended with high-resolution data to provide a service greater than the sum of its parts. By doing this, the group intends to create best-available forecasts down to the level of a single field – thus allowing farmers to decide that they should be spraying *these* fields today, but *those* fields can be left until tomorrow.

In keeping with their importance and sensitivity to environmental conditions, Dr Coop and his team will initially focus on late potato blight and spotted wing Drosophila. The predictions associated with this newly blended data will be provided to farmers across the United States, with the group holding training sessions for farmers around the Pacific Northwest area. Initial integration of the NMME models to the online portal are already popular. 'Anecdotally, they seem to make more accurate predictions than historical average temperatures, especially in the unusually warm weather we've had in recent growing seasons,' comments Nick Andrews, a researcher at Oregon State University who has been implementing the use of NMME models with fruit and vegetable growers. 'Dr Coop made access to the NMME format seamless on the IPPC websites – in usability studies people have found it easy to use and understand the new NMME forecasts.'

Forecasts for Farmers

The work being performed by Dr Coop and his fellow researchers is essential in bringing meteorological knowledge and forecasting to those who need it most – local farmers and growers. By providing detailed information on just when and where spraying should be performed, it allows for tighter control of pests with less use of insecticides and fungicides – which in turn helps both farmers and the environment. And this, in the end, helps us as we stand in the supermarket, looking at the vegetables, wondering what to eat, and checking to see whether the weather will be sunny for tomorrows' picnic.

Meet the researcher

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Dr Leonard Coop began his research career in the Entomology Department of Oregon State University, where he began to focus on pest management in commercially relevant crops. He soon moved into the field of decision support systems for Integrated Pest Management field, leading to his current dual-role as an Associate Director of the Integrated Plant Protection Center and Assistant Professor in the Department of Horticulture. With 28 peer-reviewed papers published, almost $5 million in awarded grants to date, and national leadership as director of the USPEST.ORG website, Dr Coop is a highly successful specialist within the vital field of pest management.

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PROTECTING US RICE WITH INTEGRATED PEST MANAGEMENT STRATEGIES

US rice production is threatened by the expansion of the Mexican rice borer, and a host of other pests. **Dr Michael Stout, Dr Blake Wilson** and their colleagues at Louisiana State University are investigating integrated pest management strategies to reduce the agricultural impacts of these pests.

The Mexican Rice Borer – An Emerging Threat to US Rice

The small city of Crowley, Acadia Parish, in the heart of Louisiana's rice belt is known as the 'Rice capital of America' – and deservedly so. Crowley was a major centre for rice harvesting and milling – giving the city a distinctive identity. Some of its historic mills are still operating and rice is the main crop for local farmers. Every October, the International Rice Festival is held in downtown Crowley, celebrating the harvest. With a range of fun activities – fairground amusements, live music, cooking demonstrations and the crowning of the Festival Queen – this is one weekend in Southwestern Louisiana's calendar not to be missed! The Festival is a curious hybrid of a state fair and trade convention. Tourists and locals jostle with industry officials, farmers and suppliers. Rice is big business in Louisiana, and indeed the southern US. The states of Arkansas, California, Louisiana, Mississippi, Missouri and Texas together produce 18 billion pounds annually, with 15% exported (http://www.thinkrice.com/on-the-farm/where-is-rice-grown/).

Rice is the bedrock of the local Creole and Cajun cuisines, and a steaming bowl of homemade gumbo and rice is the epitome of Louisiana's Southern hospitality. Rice is deeply entrenched in Louisiana's culture and economy – as the festivities at Crowley testify. However, the state's rice industry is incredibly fragile. Several factors have made rice production less profitable – high agricultural costs, international competition, a limited domestic market, and most recently the spread of the Mexican rice borer, an invasive pest. Many rice farmers have thus gone out of business. In fact, in the countryside around Crowley, rice fields have been repurposed as ponds for farming crawfish. Despite the decline, rice remains one of the state's top agricultural export, with an annual crop of 360 million USD.

The Mexican rice borer, *Eoreuma loftini*, belongs to the Crambidae (grass-moth) family of the Lepidoptera order of insects, which includes butterflies and moths. Adults have delta-shaped wings and a distinctive light tan colour. However, it is the larvae that are particularly worrisome for crop growers – as they feed on and bore through grass plants, including sugarcane and rice, causing great damage to crops. The adults lay eggs on dried grass leaves. Upon hatching, the larvae – distinctive with a whitish colour, light-coloured heads and purple stripes down the length of the bodies – feed on fresh leaf sheaths. They bore tunnels in the stems or stalks, filling them with frass (excrement), and then undergo metamorphosis, whereupon the young adult emerges.

Originating in Mexico, this insect has entered and proliferated in the US over the last 40 years, decimating agriculture of sugarcane, rice, sorghum and maize. The pest was detected in Rio Grande Valley, Texas in 1980, and soon became a major pest of Texas sugarcane (*Saccharum* spp.). It then spread to Texas rice, and over the past two decades, it has become a significant pest of the Texas rice industry. It has since been slowly expanding north-easterly along the US Gulf Coast. A quarantine blocking shipment of east Texas sugarcane into Louisiana was implemented in 2005 to slow the spread into Louisiana. However, even this didn't stop the invasion, and in 2008, the Mexican rice borer was detected in Southwestern Louisiana. This hardy moth can survive below freezing temperatures, and is likely to expand further north-eastwards into the rest of Louisiana, and the colder states of Mississippi and Arkansas – with huge projected economic costs. If this were to happen, losses of 220 million USD to south-eastern Louisiana's sugarcane industry are projected – unless urgent action is taken.

Integrated Pest Management for Louisianan Rice Production

Dr Michael Stout and Dr Blake Wilson, and their groups at the Department of Entomology, Louisiana State University, are experts in crop entomology. They are investigating the biology of insect pests, including *E. loftini*, and are implementing integrated pest management approaches to mitigate its damage to rice and other crops. Integrated pest management is a holistic approach to managing crop pests, geared towards sustainability and responsible stewardship of agricultural ecosystems. According to Dr Stout, 'Modern integrated pest management is (or should be) applied ecology, and good integrated pest management programs are founded upon

‘By combining research efforts in the fields of chemical and landscape ecology with applied pest management practices, we hope to enhance the sustainability of agricultural production in the US’ – Dr Wilson

a thorough understanding of the ecological interactions among the crop plant, the herbivore pest, and the other organisms such as predators or microorganisms that interact with the crop plant or the herbivore.’

The Mexican rice borer is not the only pest to damage rice in Louisiana. Established pests include the rice water weevil (*Lissorhoptrus oryzophilus*), fall armyworm (*Spodoptera frugiperda*), sheath blight (*Rhizoctonia solani*), and the sugarcane borer (*Diatraea saccharalis*) – which typically affects sugarcane but occasionally damages rice. Integration is an important component of integrated pest management programmes – such as multiple tactics against one pest, or a single tactic that impacts multiple pests. Drs Wilson and Stout and their colleagues aim to develop integrated pest management programmes that coordinate the management of Mexican rice borers, with existing tactics against established pests. Given the scale and speed of Mexican rice borer proliferation, this is a high-priority endeavour.

The team carries out their studies on rice – mainly in small plot field experiments at the LSU AgCenter Rice Research Station in Crowley and the Texas AgriLife Rice Research and Extension Center in Beaumont, Texas. These involve growing rice under carefully controlled conditions, and controlled infestations of Mexican rice borers, rice water weevils, sheath blight and sugarcane borers – to assess the influence of various factors on pest severity.

Tracking the Spread of the Mexican Rice Borer with Pheromone Traps

Out in the field, Drs Stout and Wilson are tracking the spread of the Mexican rice borer through Louisiana using pheromone traps. These traps are baited with insect sex pheromones – chemicals that female insects release into the air to attract males for mating. The team use a specific blend of sex pheromones in their traps to mimic that released by *E. Loftini*. Male Mexican rice borers mistake these traps for females and become stuck in the traps – providing an indication of Mexican rice borer populations within a region. Between 2013 and 2015, the team set up 77 pheromone traps throughout southwestern Louisiana, with male moths captured in every location – indicating that the moths are thriving here. ‘Monitoring of pest populations across large areas and applying principles of landscape ecology can improve our understanding of pest interactions and identify area-wide trends in pest population dynamics,’ explains Dr Wilson.

Pheromones can diffuse in the air over great distances – necessary for female insects to signal mating availability to faraway males. The pheromone concentration in the air decreases with distance, and beyond a certain distance, the pheromone concentration will be too low to elicit a response – a distance known as the ‘active space’. The active space is important for setting up pheromone traps. If the intertrap distance is less than the active space, then ‘trap interference’ occurs, and insects can be attracted to more than one trap, but get trapped in either one. If the intertrap distance is greater than the active space, there will be pheromone-free zones between traps where insects will not be caught. Both scenarios can reduce the accuracy of population estimation. Through field experiments, Dr Wilson determined that the active space for the Mexican rice borer is 50–100 metres. They have also carried out lab experiments involving blowing a pheromone blend towards male Mexican rice borers with high-speed fans, varying the distance between the insect and pheromone source. Under lab conditions, males showed behavioural

‘By understanding how plants respond to stresses and how they change in response to environmental conditions, we can develop ways of manipulating the expression of plant resistance’ – Dr Stout

responses from 24.5–60.6 metres, with a mean of 47.6 metres. These experiments have also provided a fascinating insight into mating behaviour. Upon detecting the pheromone, male insects were observed to pace rapidly in small circles while fanning their wings. This is consistent with observations that when seeking mating opportunities, male Mexican rice borers crawl around looking for a female, before taking flight to step up their search.

Insecticides and Fungi to Reduce Pest Damage

Insecticides are commonly used to reduce pest damage. Treating rice seeds with chlorantraniliprole before planting is now standard – and is effective against rice water weevils. Drs Stout and Wilson and their colleagues are investigating the effect of chlorantraniliprole on multiple pests out in the field. Their preliminary evidence indicates that treating seeds with chlorantraniliprole reduces sugarcane borer growth and fitness. Significantly, they have found that chlorantraniliprole seed treatment also reduces Mexican rice borer field infestations by as much as 80%. As sugarcane borers and Mexican rice borers attack later in the year, they speculate that by then, much of the insecticide will have degraded or dissolved away, and will no longer be very effective. Therefore, they hope to optimise chlorantraniliprole application against rice water weevils, sugarcane borers and Mexican rice borers.

It is not only insect pests that affect rice yields, but also microorganisms that live on and in the rice plants – bacteria and fungi. These microorganisms can be good, bad or neutral to the host plant. Arbuscular mycorrhizal fungus are soil fungi that that colonise plant roots, and form a symbiotic relationship with the plant. This fungus can help plant roots absorb more nutrients, and can, in some cases, provide tolerance against pests. An ongoing collaborative project between Dr Stout’s group and Marco Cosme at Utrecht University in the Netherlands – led by graduate research assistant Lina Bernaola, is investigating the impact of arbuscular mycorrhizal fungi on a plant’s susceptibility to insect pests (rice water weevils and fall armyworms) and pathogenic sheath blight. They found that, contrary to expectations, the fungus actually increased the plants’ susceptibility to these pests.

Chemical Ecology – Ecological Interactions Mediated by Molecules

The web of ecological interactions between organisms in an agricultural habitat such as a rice or sugarcane field is complex. Drs Stout and Wilson and their colleagues are committed to elucidating these interactions, through the field of chemical ecology – the study of how chemicals mediate the ecological interactions among organisms. ‘The study of chemical ecology is particularly fascinating because it reveals the unseen way in which organisms interact and communicate,’ says Dr Wilson.

One example of chemical ecology is induced resistance – whereby herbivory, infection, or other biotic or abiotic stresses trigger a biochemical response in a plant making it more resistant to subsequent attackers. ‘By understanding how plants respond to stresses and how they change in response to environmental conditions, we can develop ways of manipulating the expression of plant resistance,’ explains Dr Stout. When under attack by insect pests, plants can produce hormone molecules that elicit plant-defence pathways to ward off the pests – including jasmonic acid, salicylic acid and ethylene. The team uses this knowledge to develop special insecticides that mimic these natural hormones, and adjuvants that amplify their effects. They found that treatment of cotton and soybean with jasmonic acid reduced growth of fall armyworms, whereas treatment of certain elicitors, benzothiadiazole and hairpin, actually resulted in larger fall armyworms.

Out in the field, rice plants are typically attacked by multiple pests. However, it is not clear whether infestation of one pest makes rice stronger or weaker against future infestations. Herbivory can induce physiological and metabolic changes in crops – such as production of resistance-related molecules, morphological changes or changes in plant architecture – which may make plants less susceptible to future herbivory. However, this has not been extensively tested in the context of the southern US rice ecosystems. The rice water weevil is the major early-season pest of rice. Weevils live through the winter in the forests and grassy habitats surrounding rice fields. In early spring, they fly to rice fields, feed on the newly-planted rice and lay their eggs on the submerged leaves in flooded fields. After hatching, the larvae move through the leaves and stems and feed on the roots. Drs Wilson and Stout hypothesise that early-season rice water weevil infestation will induce resistance to further infestation by Mexican rice borers, and hope to confirm this. They also aim to elucidate the plant mechanisms of this induced resistance.

Drs Wilson and Stout have pioneered the application of chemical ecology, induced resistance and integrated pest management strategies as a sophisticated approach to combatting pests and improving yields. Beyond the lab, they also endeavour to apply their research insights to the field, and educate producers and industry bodies on best practices.

Meet the researchers

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Dr Michael Stout obtained his PhD in Entomology at UC Davis in 1996, for a dissertation entitled 'Induced Resistance in Tomatoes'. In 1997, he started as an Assistant Professor in the Department of Entomology, Louisiana State University, and rose to the rank of Professor in 2007. Since 1997, he has also held a position at the AgCenter (Agricultural Center) at Louisiana State University. In 2014, he became co-director of the Center for Research Excellence in Plant Biotechnology and Crop Development, and in 2016, he was appointed head of the Department of Entomology.

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Dr Blake Wilson has been working within the Rice and Sugarcane Lab in the Department of Entomology, Louisiana State University, since 2009, when he started as an undergraduate lab assistant. He obtained his PhD in Entomology in 2016, and continued his studies as a post-doctoral researcher. His special research interests include pest management strategies for sugarcane, rice, and bioenergy feedstocks. In March 2017, he became an Assistant Professor of field crops entomology with the Louisiana State University AgCenter.

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GENOTYPING OUR DAILY BREAD: GENETIC MARKERS IN MODERN WHEAT BREEDING

Wheat is a staple crop, and is of utmost importance for global food supply. **Dr Guihua Bai** and his group at USDA-ARS conduct wheat genomics research to analyse wheat DNA markers, and assist in the breeding of new cultivars, to ensure high grain yields and quality, as well as resistance to multiple biotic and abiotic stresses.

Norman Borlaug (1914–2009), American agronomist and father of the Green Revolution, saw food security as a global priority, stating, 'Without food, people perish, social and political organisations disintegrate, and civilisations collapse.' Raised in rural Iowa by a Norwegian-American farming family during the Great Depression, Borlaug was all-too familiar with the socio-economic implications of food supply, and the inefficiencies of the standard agricultural practices of his day. Borlaug went on to meticulously cross-breed thousands of wheat varieties to produce advantageous traits such as high yields, disease-resistance, early maturation, dwarfism and tolerance to environmental conditions. Borlaug's pioneering programme kick-started the Green Revolution that saw global crop yields skyrocket. The productivity gains from Borlaug's agricultural innovations are thought to have saved over a billion people from starvation, as well as preserved large tracts of pristine wilderness from being converted to farmland. It was not for nothing then that Borlaugh was awarded the Presidential Medal of Freedom, the Congressional Gold Medal, and the Nobel Peace Prize, being one of only six people in history to receive these three prestigious awards.

Borlaugh's focus on wheat production to ensure food supply and security was well justified. Wheat is a staple food source for billions, with 729 million tonnes produced worldwide in 2014. While Borlaugh's efforts were revolutionary, further innovations in wheat breeding and production are necessary to feed a growing human population. But first, it is essential to understand the evolution and genetics of modern wheat varieties in order to evaluate the complex interplay between genotype, phenotype and environment.

The Complicated Phylogeny of Wheat

Cultivation of wheat (*Triticum* spp.) is thought to have started in the Fertile Crescent around 9600 BC and spread worldwide. Due to domestication and selective breeding of wheat, modern wheat varieties bear little resemblance to their wild ancestors, having ears that hold more grains, as well as greater tolerance to environmental conditions and features amenable to modern farming practices. Dramatic increases in genome size are a characteristic feature of the grass family (*Poacea*), of which wheat is a part of. Therefore, cereal crop plants are typically polyploid, having more than two pairs of homologous (pairing) chromosomes.

Most cultivated varieties of wheat belong to two species, the durum wheat (*Triticum durum or Triticum turgidum* subsp. *durum*) used in pasta and semolina, and bread wheat (*Triticum aestivum*). The phylogeny of both common domesticated wheat species is somewhat complicated by a series of genome duplications, hybridisations between species and trait selection. Around 7 million years ago, an ancestral *Triticeae* gave rise to two divergent diploid precursor genomes, denoted AA and BB. Then, about 5 million years ago, these formed the diploid DD genome (2n) of *Aegilops* genus (goatgrass). About 800,000 years ago, tetraploid wild emmer wheat appeared (*Triticum turgidum*), formed from a hybridisation between ancestral wheats with AA and BB genomes. The domestication of emmer wheat reflects the dawn of agriculture, as hunter-gatherers began deliberately cultivating wild emmer wheat. The selection of these first farmers resulted in plants with larger grains, giving rise to domesticated emmer wheat (*Triticum turgidum* subsp. *dicoccum*). Further selection produced durum wheat (*Triticum durum or Triticum turgidum* subsp. *durum*) around 9,000 years ago, the only tetraploid species of wheat of commercial importance that is widely cultivated today. Expansion of emmer wheat cultivation into wild areas led to chance of hybridisation between domesticated emmer (AABB) and wild goatgrass (DD), producing today's hexaploid bread wheat (*Triticum aestivum*) (AABBDD), with kernels that can easily be released from the spike, allowing free-threshing.

The Quest for Wheat Genetic Markers

Dr Guihua Bai from USDA-ARS is passionate about wheat genetics, and considers this important for the future of wheat cultivation. He is the principal investigator in the USDA Central Small Grain Genotyping Center in Manhattan, Kansas and provides DNA marker service to breeders in the hard winter wheat regions in the US Great Plains. His group applies state-of-the-art modern genotyping techniques to discover and validate wheat DNA markers that are subsequently used to select important traits. The genotyping data can then provide selection guides to wheat breeders seeking to create new cultivars in response to specific challenges, such as newly emergent plant pathogens or climate change. This is no easy feat, given the size and complexity of three sub-genomes of bread wheat.

The typical genome of *Triticum aestivum* is composed of 17 Gbp (giga-base pairs) of DNA, of which 80–90% is made of repetitive sequences, presenting an enormous challenge for DNA sequencing and analysis. The partial draft sequence of the *Triticum aestivum* genome in 2010 by a group in the UK represented a milestone in wheat genomic research. However, a recent coordinated international endeavour (http://www.wheatinitiative.org/) on subsequent re-sequencing, sequence re-assembly, and gene identification and annotation, gave birth to a relatively complete wheat genome reference sequence. The newly assembled reference sequence provides critical resources for gene cloning, DNA marker identification, and elucidating the genotype-phenotype relationships of agriculturally relevant varieties that Dr Bai focuses on.

The analysis of DNA markers lies at the heart of Dr Bai's research. Of particular interest to his team is the analysis of quantitative trait loci (QTLs) or genes that are important to wheat production. A QTL is a DNA section (a locus), usually containing one or more genes, responsible for a particular quantitative phenotypic trait. Genes are DNA sections encoding different amino acid sequences that make up proteins, which, at the molecular level, are ultimately responsible for determining physiological phenotypes. In most of the cases, the identity of gene(s) underlying a QTL is difficult to discover, but the DNA fragments nearby the gene can be easily identified and serve as landmarks for selecting the gene(s) in breeding. These polymorphic DNA markers physically close to genes of interest on a chromosome are more likely to be inherited together with the gene (DNA sections are 'shuffled' during meiosis) – this is known as genetic linkage. While most QTLs are postulated to have a minor effect on plant physiology, Dr Bai's group seeks to identify the ones that have major and stable effects using DNA markers.

In traditional breeding, breeders directly select the trait of interest, and these selections need to be done under certain environments. For example, selection for disease resistance has to be done where the plants are severely infected by the disease, whereas high yield traits have to be selected under disease free conditions. New cultivars must show high yields and good quality, and also resistance to multiple diseases, each of which needs to be selected in a different environment in traditional breeding. Using DNA marker-assisted selection (MAS), these traits can be selected by testing a small piece of leaf at the early seedling stage to predict presence of target genes or QTLs, significantly reducing phenotyping costs, speeding up the breeding process, and increasing selection accuracy. Dr Bai's group provides marker analysis services to breeders in the US Great Plains to help them deploy MAS in US hard winter wheat breeding.

As new generation sequencing (NGS) technologies, DNA marker technologies have been significantly improved. SNPs (single nucleotide polymorphisms) including insertions/deletions ('indels') are common polymorphic markers for genotyping. Polymorphic SNP markers can be easily found by comparing DNA sequences between genotypes. Recently, Dr Bai and his team found that a single SNP tightly linked to a major QTL on chromosome 7A of *T. aestivum* had a major impact on kernel

Wheat with a scab resistance gene, Fhb1, transferred by marker-assisted selection (right) and without Fhb1 (Left)

Wheat with TaPHS1 (left) and without TaPHS1 (right)

length and weight. They did this by employing an SNP Chip – a DNA chip technology that can simultaneously identify a large number of DNA polymorphisms for QTL identification. They then converted the chip-based SNP to a breeder-friendly marker type called KASP assay, a PCR-based technique involving the use of allele-specific primers and fluorescent probes, so breeders can use this KASP-SNP marker to directly predict the presence of the allele of interest. The group has recently demonstrated the use of NGS for low-cost multiplex analysis of several polymorphic markers associated with various important wheat traits, including disease and insect resistance, grain quality and plant height, which significantly reduced genotyping cost and increased efficiency.

Genomic Approaches to Tackling Pre-Harvest Sprouting

Long seed dormancy (SD) is advantageous to wild grasses in a harsh environment, allowing germination only under optimal conditions. This is a feature that has been 'bred out' of modern wheat varieties by artificial selection, as short and predictable germination is conducive to high productivities and straightforward breeding. However, a major disadvantage of short SD is propensity for pre-harvest sprouting (PHS), which involves premature germination of kernels in a spike before harvest. PHS is problematic for farmers as it causes significant losses in yields and grain quality. In regions with high rainfall during harvest season, the cultivation of wheat cultivars with appropriate SD and resistance to PHS is advocated. Identification of alleles and QTLs associated with PHS resistance or susceptibility, to inform breeding strategies, is a high priority research theme within the USDA Central Small Grain Genotyping Center.

TaPHS1 is a gene known to regulate PHS resistance on chromosome 3A in wheat. Dr Bai's group cloned the gene and identified two mutations in the positions +646 and +666 of the *TaPHS1* coding region that result in wheat PHS susceptibility in a white wheat Rio Blanco. Dr Bai's group screened 327 wheat accessions of wild and domesticated wheat progenitors with A genome using three KASP markers based on the two *TaPHS1* mutations and one mutation in the promoter region. The wheat species were also assessed for PHS tolerance under greenhouse conditions, to correlate the genotype to the phenotypic trait. It was found that most accessions of wild wheat progenitors were highly PHS-resistant, and all lacked the +646 mutation. Hexaploid wheat and *T. durum* were found to have the highest sprouting rates, demonstrating the diversification of SD during domestication. Interestingly, it was found that +646 mutation occurred independently in *T. monococcum* and *T. aestivum*, driven by the same selection pressure for reduced seed dormancy.

Plant breeders and farmers alike have sought to determine quantitative indicators of PHS susceptibility. What better indicator than grain colour (GC)? Colorimetric analysis is intuitive and amenable to straightforward observation without specialist equipment. Previous anecdotal and empirical observations have suggested that GC is associated with PHS, with red-grained wheats more tolerant to PHS than white-grained wheat. Dr Bai's group have confirmed this relationship between GC and PHS tolerance with a genome-wide association study (GWAS) in 185 elite lines and cultivars. For these cultivars, both sprouting studies in greenhouses and field experiments, and genotyping studies were carried out with wheat 9K and 90K SNP arrays. The GWAS study found that a number of genes, mainly on group 3 chromosomes, that control GC also regulate PHS resistance, but this relationship was observed in the field but not the greenhouse, highlighting the importance of environmental conditions on triggering gene-encoded phenotypes. In addition, several genes that do not relate to GC were also identified, which are important for breeding PHS tolerant white wheat.

Marker-Assisted Selection in Wheat Breeding – The Way Forward

In a sense, Dr Bai is following in the footsteps of Norman Borlaug, who began a revolutionary wheat breeding programme 50 years ago. However, plant breeding techniques have moved on, with new genomic tools and approaches that would have been unknown to Borlaugh in the 1960s. Dr Bai and his group deploy the latest genomic technologies to analyse QTLs and associated DNA markers, to probe the complex relationships between genotype and phenotype in wheat. They then utilise these insights to inform breeding strategies, with the aim of producing elite cultivars enriched with advantageous QTLs, while minimising deleterious QTLs. This is no easy task, given the complexities of gene segregation, and the reduced gene pool from millennia of selection. It is hoped that marker-assisted selection, coupled with innovations in agricultural practices, will fulfil the original vision of Borlaug's Green Revolution and further boost productivities to feed an ever-growing human population.

Meet the researcher

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USDA Agricultural Research Service

IMPROVING PLANT DISEASE RESISTANCE: CAN NANOPARTICLES DELIVER?

Crop disease can have an enormous impact on farming productivity and profits. A plant's resistance to disease has been previously linked to micronutrients, which control key biochemical processes. **Dr Wade Elmer** of the Connecticut Agricultural Experiment Station has carried out research showing that nanoparticles can provide an effective, practical method of getting vital micronutrients such as copper into crops.

The Challenge of Keeping Plants Disease-Free

Managing nutrition in any organism is fundamental for its health and ability to resist disease. In plant pathology, three key factors are recognised as important drivers behind disease development. These factors are the presence of the pathogen, the environmental conditions that allow the infection to thrive, and the susceptibility of the plant itself to the disease. Altering any one of these three things will affect how severely the plant is infected, or whether it becomes infected at all.

One way that this 'disease triangle' can become altered is through environmental change – for example, increasing the soil's moisture content can make certain pathogenic organisms more aggressive. Plant pathologists work to reduce the probability of plants becoming vulnerable to disease, by looking at ways to positively influence one or more of the three parts of the disease triangle. Sometimes this involves trying to reduce the presence of pathogens through chemical or biological treatment of the soil. Other methods include figuring out how to make the plant environment less conducive to infection taking place, by altering moisture content, temperature or light levels.

Some plant pathologists, however, such as Dr Wade Elmer of the Connecticut Agricultural Experiment Station, examine ways to improve plant nutrition to reduce disease susceptibility. Poor plant nutrition, where the soil is low in vital nutrients, is a well-known factor that can lead to reduced resistance to disease. In these nutrient-poor regions, plants cannot maintain a healthy metabolism and can often become vulnerable to infection. For Dr Elmer, his work is a something of a family tradition. 'My father was an entomologist and my grandfather was a plant pathologist so I always had exposure to plants and agricultural research,' he explains.

Just as with different animal species, each type of plant requires a different diet of nutrients, comprising nitrogen, potassium, phosphorus and many other elements in greater or lesser amounts. For agricultural crops, there is a strong financial incentive to obtain the perfect balance in order to achieve profitable yields. Therefore, scientists like Dr Elmer spend long hours setting up experiments and working out the optimal nutritional balance for individual crops and crop varieties.

In 2015, Dr Elmer published a fact sheet on the effects of mineral nutrition on plant health, specifically focussing on disease suppression in agricultural crops. In this article, he demonstrated that several elements are vital for improving plant resistance to disease, and also showed that for each of these elements, a number of different metabolic pathways are important. Within the complex biochemistry taking place in a plant, Dr Elmer showed that micronutrients – elements that are present in low concentrations but are vital for specific processes within the plant's cells – can play pivotal roles in the plant's ability to resist infection by a number of diseases.

Delivering Micronutrients with Nanoparticles

Multiple methods exist for supplying micronutrients to plants, some of which are more effective than others. Recently, within the agricultural science community, there has been much excitement surrounding the potential for using nanoparticles as a micronutrient delivery system. Nanoparticles are particles of extremely small size – on the order of billionths of a metre in diameter, much too small to be seen even with an optical microscope. Often, micronutrients cannot readily move through the soil and become tightly bound to soil particle surfaces, making them unavailable to plant roots. However, nanoparticles are often much more mobile and accessible, allowing them to be taken up and used by plants much more effectively.

In research published in *Environmental Science Nano* in 2016, Dr Elmer and his colleague Dr Jason White, investigated the possibility of improving plant disease resistance by enhancing their uptake of

‘Just as people who do not take care of themselves with proper nutrition are more susceptible to disease, plants can also be more vulnerable to infection when their nutrition is not optimal’

The effect of CuO nanoparticles on watermelon plants infected with Fusarium wilt

different micronutrients using nanoparticles. The team sprayed solutions of six different types of metal oxide nanoparticles onto the leaves of tomato and eggplant seedlings, which were growing in a greenhouse. These plants were then grown in soils or soilless medium, which had been deliberately cultured with Fusarium wilt fungus or a Verticillium wilt fungus – common pathogens known to infect them.

After three weeks, the leaves of the plants were examined for signs of disease. The team also recorded the number of fruit growing on the tomato plants, and measured their weights, and assessed the size of leaves on the eggplants. Of the six types of metal oxide nanoparticles tested, they found that those containing copper, manganese and zinc all reduced the presence of disease, while copper in particular boosted crop yield for both tomatoes and eggplant.

The next step in this experiment was to investigate *how* the copper nanoparticles were helping the plants to resist disease. Dr Elmer wanted to find out whether the copper was directly influencing the fungal disease, or if it was having an effect on the plant’s overall health and disease resistance. If it was directly inhibiting the disease, then applying nanoparticles to the soil where the disease was present would make more sense than spraying them onto the plants’ leaves. However, if the copper was somehow enhancing the plants’ health and disease resistance, then applying the nanoparticles onto the leaves would be the most effective option.

An analogy to consider is the way we try to avoid getting the flu – we can either sterilise

infected surfaces to lessen our chances of coming into contact with the virus, or bolster our immune systems so that we can fight off the infection when it occurs. What Dr Elmer discovered was that the copper did not directly attack the disease in the soil. Instead, it appeared to be taken up by the plant and used to increase disease resistance.

When they cut up the copper-treated plants after the experiment, Dr Elmer and his team also found that the roots contained increased levels of this micronutrient. This suggests that the nanoparticles can move within the plant from the leaves down into the roots, where they are used to boost plant health and disease resistance. During the experiment, the team had taken care not to spray any of the nanoparticle solution onto the soil or soilless medium. Therefore, they were confident that the nanoparticles had not been directly taken up by the roots, but were instead transported through the plant from the leaves.

Implications for Agriculture

Due to their tiny size, nanoparticles of copper and other elements behave much differently to larger particles. They are much more mobile, allowing plants to easily absorb them. A side-effect of this is that too much of one element can produce toxic effects within the plant. Additionally, plants containing high levels of certain elements can potentially be harmful to the people that ultimately consume them. Copper in particular can cause problems if too much is supplied to the plant – it can cause direct toxic effects as well as preventing other vital micronutrients from being taken up by the plant.

Through the use of nanoparticles, Dr Elmer's team were able to deliver very small amounts of copper to young plants – enough to improve their disease resistance without inducing toxicity in the plants, or creating levels unsafe for human consumption. Even in relatively small amounts, these nanoparticles are taken up so effectively by the leaves that they are much more efficient than larger particles.

Another important result from the team's experiments was that the effects of a single dose lasted a long time, often throughout the entire growing season. This means that a small dose early on in the plant's life might be enough to maintain disease resistance all the way to harvesting time. If this is true, then micronutrient dosage could be kept smaller, enabling farmers to grow food safely with improved yields and less disease risk. This would also mean lower amounts of these elements released into streams and waterways, therefore reducing environmental impact.

Future Work

Dr Elmer plans to carry out further work to explore the ways in which plants take up nanoparticles and how they transport them from the leaves to the roots. He wants to particularly focus on pathways that have been identified as important for plant health and disease resistance. This is an important question, as applying these micronutrients in other non-nanoparticle forms do not have the same effect – and researchers have typically struggled to get micronutrients to move from the above-ground part of the plant down into the roots.

Dr Elmer also wants to find out whether these nanoparticles have the potential to cause unsafe micronutrient levels in the fruits of tomato and eggplant crops. This will involve exploring the processes that are involved in nanoparticle absorption and movement throughout the plant. His team have already found a way to boost micronutrient concentrations in the roots and leaves of the plants, while leaving the fruits unaffected. How and why this happens is not yet clear, and Dr Elmer is keen to explore further.

If an explanation can be found for why the fruits maintain normal levels of micronutrients, then this will be able to reassure farmers and customers that nanoparticle application of micronutrients is completely safe. Moreover, if Dr Elmer and his team can solve the problem of making micronutrients available to the right part of the plant, then this will open the door to further questions that need answering in order to improve the use of nanoparticles for plant disease resistance.

Dr Elmer also plans to investigate the best way to apply these micronutrient nanoparticles, and the optimal amounts to use for each crop species. Finally, his team also hopes to identify the mechanisms responsible for the plants' improved disease resistance, and currently Dr Elmer is trying to work out if (and how) the copper nanoparticles help the plant produce chemicals in its cells that are harmful to disease.

Meet the researcher

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Dr Wade Elmer obtained an MSc in Plant Pathology at VPI&SU in 1981 and a PhD in Plant Pathology at Michigan State University in 1985. He then joined the Connecticut Agricultural Experiment Station in 1987 as an Assistant Scientist, where he has now been working for the last 30 years. Since 2015, Dr Elmer has been the Chief Scientist of the Department of Plant Pathology and Ecology at the Connecticut Agricultural Experiment Station.

Dr Elmer has been a member of the American Phytopathological Society (APS) since 1979, and has held several committee roles in the Northeast Division of the Society, including President in 2006. He also acted as an editor on several crop disease journals, including Plant Disease, Crop Protection and Phytopathology. He was awarded the CHOICE Award for Best Outstanding Academic Title in 2008 as an author for Mineral Nutrition and Plant Disease (APS Press, St. Paul, MN).

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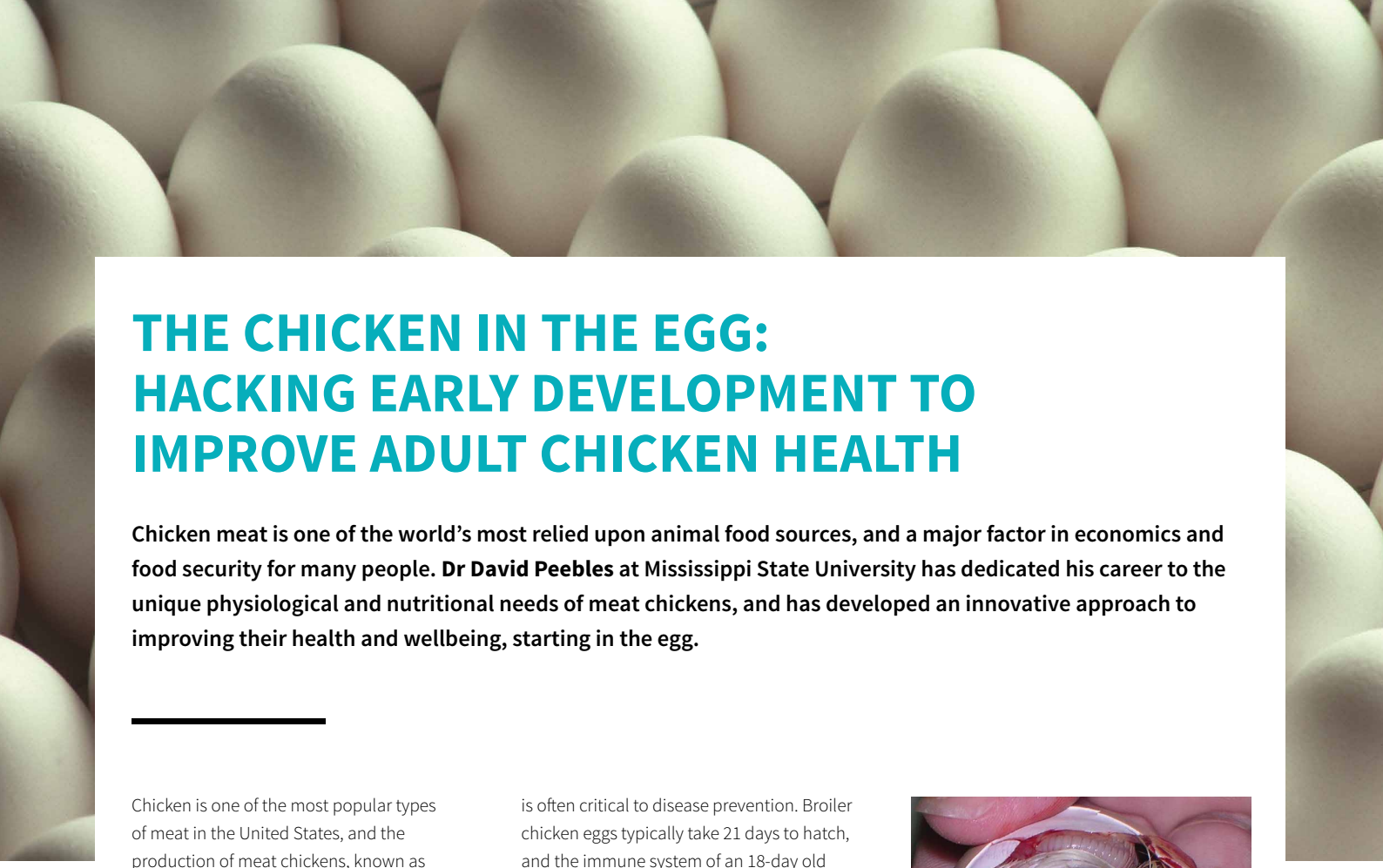


THE CHICKEN IN THE EGG: HACKING EARLY DEVELOPMENT TO IMPROVE ADULT CHICKEN HEALTH

Chicken meat is one of the world's most relied upon animal food sources, and a major factor in economics and food security for many people. Dr David Peebles at Mississippi State University has dedicated his career to the unique physiological and nutritional needs of meat chickens, and has developed an innovative approach to improving their health and wellbeing, starting in the egg.

Chicken is one of the most popular types of meat in the United States, and the production of meat chickens, known as broiler chickens, is a major agricultural industry. The U.S. alone produces about 8.6 billion broiler chickens every year for approximately $45 billion in sales. Supporting the health and wellness of these animals is of paramount concern, both from animal welfare and economic perspectives. Processes and procedures that help to improve the health of chickens before they even hatch can have dramatic effects for chicken wellbeing and farmer profits. Dr David Peebles studies chicken physiology from his laboratory at Mississippi State University, focusing on interventions such as vaccines and supplements that can be supplied to young broiler chicks before they even hatch, preventing disease and supporting healthy growth and development. His work helps broiler chicken farmers raise happier, healthier flocks and reduce losses due to disease and development abnormalities.

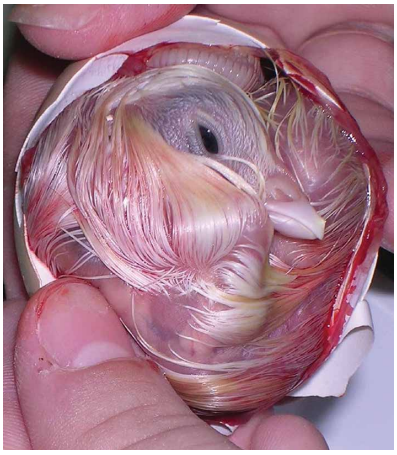
Shifting Best Practices in Poultry Disease Prevention

Many avian diseases can be devastating to a flock of susceptible birds, and due to their genetic similarities, broiler chickens are often hit particularly hard by outbreaks. Disease outbreaks cause suffering for the chickens, and can be devastating to farmers when diseases are deadly. Many diseases can affect newly hatched chicks, so early vaccination is often critical to disease prevention. Broiler chicken eggs typically take 21 days to hatch, and the immune system of an 18-day old embryo growing inside an egg is developed enough for a vaccine to be effective.

Vaccinating embryos a few days prior to hatching gives young chicks the opportunity to develop immunity to diseases while they are still safe inside their shells, and has been transformative for helping broiler chicken farmers prevent disease. The practice of pre-hatch or '*in-ovo*' vaccinations has grown widespread in the poultry industry; however, new vaccinations must be carefully tested to ensure that they do not negatively impact growing embryos nor affect the health and body size of adult birds. Dr Peebles and his team set out to test the effectiveness and long-term health effects of pre-hatch vaccinations for common broiler chicken diseases.

Marek's disease is a serious avian viral disease that often leads to paralysis and death in chickens. An outbreak of Marek's disease can lead to massive losses of life, with death rates of up to 80%, so vaccinating against this virus is critical to protect broiler flocks. Traditionally the Marek's disease vaccine has been administered subcutaneously to newly hatched chicks, but recently *in-ovo* injections have become more common.

Dr Peebles and his team wished to test the long-term effects of *in-ovo* vaccinations on



chicken growth, and see if the stress of either mode of injection might interact with other stressors, such as staying in the incubator for a long period after hatching before being moved to a pen. They vaccinated one group of broiler chicken embryos *in-ovo*, and another group of newly hatched chicks

subcutaneously. From each vaccination group, half of the chicks were left in their hatching incubator for a standard 4 hours, while the other half stayed for 18. They then monitored the chicks regularly until they reached adult weight (about 7 weeks of age). When comparing adult birds, they found no effect of vaccination protocol, but birds from the 18-hour incubation groups showed slower growth. These results demonstrate that the *in-ovo* vaccinations were not detrimental to adult health.

The parasite coccidiosis is another critical avian disease that many farmers opt to vaccinate their chickens against. Mild coccidiosis causes weight loss and poor digestion, while more severe cases can lead to death, particularly in chicks. The vaccine for this parasite is a live non-attenuated dose of coccidiosis eggs, and concerns existed that the vaccine might reduce weight gain in growing broiler chicks. Dr Peebles and his team compared *in-ovo* vaccinated chicks to unvaccinated and saline injected peers, and found no detrimental effect, indicating that the vaccine is safe and beneficial for use in broiler chickens.

Broiler Health Begins in the Egg

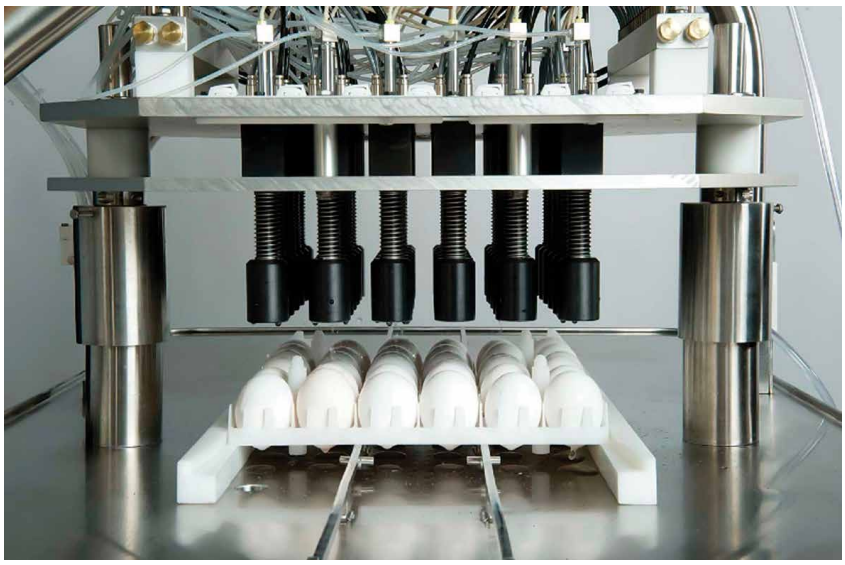
Broiler chickens have been specifically bred over many generations to grow much faster than other types of chicken, and have larger muscle mass in areas of the body that are used for meat cuts. Beyond infectious avian diseases, broiler chickens are susceptible to a number of health issues related to this rapid growth. Many of these conditions begin early in life, and some are reversible or can be moderated by nutrition.

Dr Peebles' early career research focused largely on hen and chick nutrition, infectious avian diseases, and egg incubation conditions, which many of his graduate research students still engage in. However, his focus has shifted slightly over the past five years. He recognised that over 95% of commercial broiler chicken producers were already engaged in injecting *in-ovo* vaccinations, and thought that perhaps the same procedure could be used to introduce nutrients, electrolytes, and other compounds that could contribute to the health of chickens and support their fast growth. Many of these compounds can be administered at the same time as vaccinations, reducing the need for multiple injections into the egg, and potentially providing young chicks with a head start on a healthy life.

Unlike mammalian mothers, who are able to provide their growing embryos with nutrients almost continuously, a hen must deposit all the nutrients a growing chick needs at the time she lays the egg. Healthy mothers are more likely to lay healthy eggs, but even in the best of situations, a mother hen laying multiple eggs may not deposit nutrients equally between eggs, putting some of her offspring at a potential disadvantage. These small changes are minor in most chickens, but in rapid growing broilers they can quickly lead to health problems, such as low hatch rates, bone abnormalities, and poor post-hatch growth. *In-ovo* injections could grant farmers the ability to level the playing field and hatch healthier, more robust chicks. However, this use of the technology is still in its infancy and the effects of many potential nutrients and compounds are not yet well known. Dr Peebles and his team have engaged in numerous *in-ovo* nutrient studies, illuminating potentially helpful compounds that can supplement maternal nutrients for stronger chicks.

Supplements for Chicken Health

Supplementing embryos before they hatch requires a delicate understanding of the physiology of a developing chick, as many compounds that an adult bird could happily eat might not have the same effects inside the egg. It is critical to identify what compounds have the potential to be used to the chick's benefit, versus those that are likely to cause damage. One of the first nutrients



that Dr Peebles studied as a supplement to include alongside *in-ovo* vaccinations was L-carnitine. L-carnitine has been shown to increase muscle mass and decrease fat when added to chicken diets. Although adult chickens can produce this compound naturally, embryos have limited ability to produce it in the egg, despite some evidence that it might help embryos extract nutrients more easily from their yolk.
Dr Peebles' team added different levels of L-carnitine to 18-day old eggs and observed subsequent hatch rates and adult body condition. Although the compound did not change adult body composition, it led to minor health improvements in newly hatched chicks, and at high levels increased successful hatching rates, making it an excellent target for supplementation programs. Similarly, the team tested a range of common electrolytes at different concentrations. They found that potassium chloride and tripotassium citrate both hold promise as electrolytes that can improve hydration in the egg, allowing chicks to hatch in better health.

While some nutrient supplements showed promise, others required more caution. Dr Peebles tested injections of various carbohydrate solutions, in the hope of providing an additional energy source for the developing chick. One of the most common measures to assess the quality of newly hatched chicks is body weight, and the gold standard is yolk-free body weight, when the leftover yolk is removed prior to weighing. Though more labour-intensive, this provides a clearer picture of how developed the chick is – the yolk is the only food source in the egg, so a chick with low yolk-free body weight and lots of leftover yolk is likely less developed upon hatching, compared to a chick that has consumed its yolk. When total hatchling body weight is taken without cleaning off leftover yolk, this difference is often concealed.

Dr Peebles' team found that chicks that had been supplemented with any type of carbohydrate had a higher total body weight at hatching. However, chicks that had received maltose, sucrose, or fructose had lower yolk-free body weights than their peers who had received glucose or dextrin, suggesting that some carbohydrates interfere with yolk absorption during development. Farmers should use care if utilising these carbohydrates with their *in-ovo* supplementation regiments.

One of the team's most promising findings has been the effects of vitamin D3 when provided to developing chicks pre-hatch. Due to their accelerated growth and development, broiler chickens often have weak, flexible bones and are prone to bone abnormalities and injury. Adding a moderate dose of vitamin D3 with the vaccine injection dilution improved bone development, and a larger dose significantly improved bone mineralisation and health. Further, the vitamin alleviated one of the primary detrimental effects of *in-ovo* vaccination – slightly lower hatch rates – and improved the calcium balance in the yolk. The benefits of *in-ovo* supplementation with vitamin D3 are very high, improving both hatch rates and the overall health and robustness of adult birds.

New Frontiers in Ancient Ways

As technology advances, even age-old traditions like raising chickens benefit from the incredible improvements that science offers. The practice of vaccinating chicken embryos while they are still developing in the egg has dramatically reduced incidence of avian infectious diseases and helped to protect flocks from potentially devastating outbreaks. As this practice becomes increasingly widespread, savvy physiologists like Dr Peebles and his colleagues have been able to use their expert understanding of the needs of developing embryos, to co-opt the vaccination procedure and provide additional nutritional support to growing chicks, before they even hatch. The result is healthier chickens that require less medical intervention as adults and experience more comfortable lives, all while increasing yields for chicken farmers. Dr Peebles and his team will continue their work identifying beneficial pre-hatch compounds and using innovation to improve chicken health worldwide.

Meet the researcher

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Dr David Peebles began his research career at the University of South Carolina with a Bachelor's degree in Marine Biology in 1979, and continued his education with a Master's in Biology from the College of William and Mary in 1981. He went on to obtain his PhD in Physiology from North Carolina State University in 1986, with a dissertation on broiler chicken egg production, before founding his current laboratory in the Department of Poultry Science at Mississippi State University. He currently serves as a professor of Poultry Science, splitting his time between teaching undergraduate genetics and performing innovative research. He has trained over 30 Master's and PhD students, and mentored many undergraduate researchers. He has devoted his academic career to understanding and improving the health and physiology of broiler chickens through nutrition and management practices. His work is widely influential in the poultry farming community, and his methods continue to improve poultry health and yields in the United States.

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GLOBAL HEALTH IS FOR THE BIRDS: STUDYING AVIAN DISEASES TO PROTECT HUMAN HEALTH

Every year, outbreaks of diseases that affect livestock cause massive economic losses, and occasionally these diseases also threaten human health. The dynamics that influence the emergence and transmission of these diseases are complex, and studying them effectively requires collaboration between scientists from many disciplines. **Dr Chang-Won Lee** and his multidisciplinary research team partner with farmers, scientists, and governments around the world to develop ways to understand, predict, and prevent diseases in all species.

Among the greatest threats to global human health are animal borne diseases that gain the ability to infect humans, known as zoonotic diseases. Many of our worst modern diseases originated in other species and later jumped to humans, including the Ebola virus, HIV and Salmonella. In recent years, particularly strong variations of avian and swine flu have given doctors and scientists cause for concern.

Zoonotic diseases that develop in livestock are often the most likely to affect human health on a large scale, as farm raised animals come in direct contacts with farmers, food suppliers, and eventually consumers. Further, even if a disease does not gain the ability to infect humans, diseases that affect these animals can lead shortages in the food supply and drastically impact the quality of life of farm animals. Dr Chang-Won Lee has devoted his career to studying zoonotic and animal diseases, and is leading a team of scientists to tackle some of the biggest challenges in human and livestock health.

Connecting the Dots to Fight Emerging Diseases

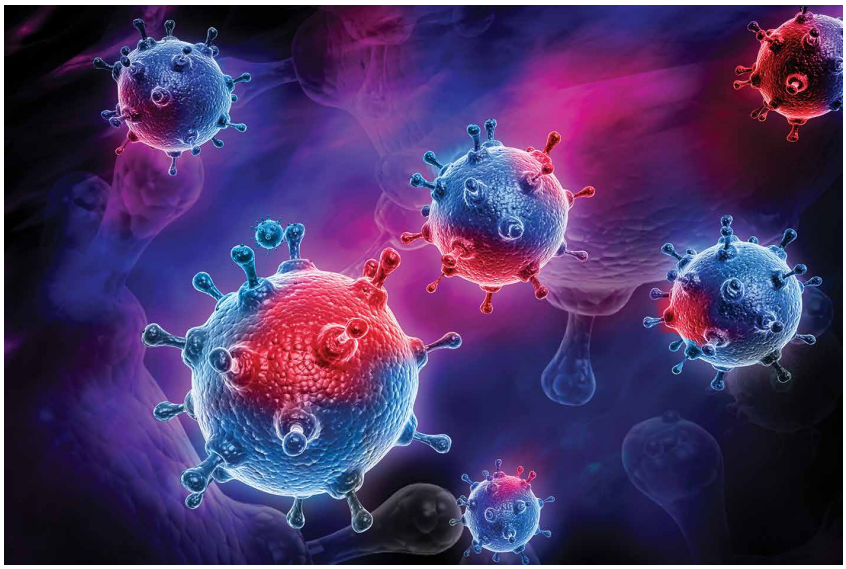
One of the most critical components of managing a disease outbreak is early identification and intervention. Success in containing a would-be zoonotic outbreak depends on accurate detection of a disease, and well-coordinated communication between farmers and health agencies. While the United States has numerous programs in place to track and contain the emergence and spread of diseases that primarily impact humans, similar networks for livestock diseases are lacking. Outbreaks of zoonotic strains of Highly Pathogenic Avian Influenza (the bird flu virus) in 2015 and 2016 have spurred researchers and policy makers to address this gap. These outbreaks pose a risk not only to human health, but also to the economic security of local farmers, as these diseases can lead to massive suffering and loss of life among flocks of poultry. The management and prevention of an outbreak ensures food security and maintains healthy and humane conditions for avian livestock.

The Poultry Respiratory Disease Coordinated Agricultural Project (PRD-CAP) is a collaboration between eleven academic institutions in the U.S., aimed at developing research-backed approaches to the prevention and control of respiratory diseases in avian livestock. The project, funded by the United States Department of Agriculture (USDA), encompasses three major areas: understanding the ecology and transmission of poultry respiratory diseases, understanding the factors that contribute to an outbreak, and developing novel, broad spectrum vaccines for diseases that impact poultry.

In addition to research in these areas, the PRD-CAP identifies and provides financial support to researchers with initiatives that benefit the project's cause, such as a recently funded project developing an educational video series and research into the transmission dynamics unique to backyard chickens. Dr Lee is at the head of the primary PRD-CAP initiative, working to illuminate the biology of avian respiratory diseases and improve the ability of farmers, scientists and government agencies to identify and respond to an outbreak. His team is laying the groundwork for a nationwide system to both track the emergence of avian respiratory diseases, and educate farmers in disease identification and management to help prevent diseases from spreading through their flocks and to other farms.

Similar to most poultry producing states, the poultry industry in the state of Ohio comprises three separate segments: broilers (chickens raised for meat), layers (chickens raised to lay eggs), and turkeys. Connecting these segments of the poultry industry could prove essential during an outbreak,

Dr Lee has been studying transmission of the flu within and between species, and developing universal flu vaccines and vaccine formulations that could work on multiple species.



as communication between farmers who do not normally interact could prevent the spread of a disease across multiple farms and species. Dr Lee and other scientists including extension veterinarian at the Ohio State University (OSU) is partnering with the Ohio Poultry Association, Ohio Department of Agriculture, and the poultry industry to develop the Ohio Poultry Network and Disease Control Committee.

The goal of this committee is to increase connectedness and communication between the three segments of the Ohio poultry industry, provide a centralised data repository for state disease information, develop action plans for committee members to follow in an outbreak, and to provide educational materials and support to poultry farmers. During the last two outbreaks of Highly Pathogenic Avian Influenza, the OSU group produced educational materials including articles and a brochure, and provided in person educational sessions to inform local poultry farmers about the disease, and ways to manage their flocks to reduce harm caused by the virus. The brochure has now been distributed widely in Ohio, providing critical information to people at all levels of the poultry industry.

Thanks to collaborative efforts among industry, government and the university, the majority of Ohio poultry owners are now prepared to send samples to diagnostic laboratories at the first sign of symptoms. Future goals include extending outbreak education to support farmers on what to do in the event of a severe outbreak that requires more drastic intervention. The group hopes to develop and maintain a digital map of the poultry farms in Ohio, which can be layered with the data being collected about incidence of respiratory disease. The group is also working to implement and extend similar programs in other states that still lack a poultry disease tracking network, and now holds a presence in the majority of poultry producing states. The ultimate goal is a nationwide network that can quickly and effectively identify and contain potential poultry respiratory disease outbreaks before they spread to multiple farms and devastate local economies.

The Avian Microbiome and Disease Prevention

In recent years, interest in the trillions of microorganisms that naturally live in and on our bodies has skyrocketed. Known as the microbiome, the average human carries more bacterial cells than human in their body, and researchers are beginning to recognise the incredible role these tiny organisms play in maintaining our health and wellbeing. Over the past decade, microbiome research has exploded as improved technologies make it possible to identify bacterial species more easily and accurately. An important component of understanding disease ecology in poultry involves understanding the natural viral and bacterial flora present in healthy birds. A second arm of Dr Lee's research in the USDA-funded PRD-CAP is illuminating the components of a healthy poultry microbiome between commercially raised broilers, layers and turkeys.

Dr Lee's research group in Ohio is currently engaged in describing the microbiome of turkeys. In the initial phase of their study, they aimed to describe changes in the turkey microbiome from hatching to adulthood, and microbiome similarities between turkeys of the same age and parts of the body sampled. They used sinus washes and tracheal washes

to collect bacterial samples from the respiratory tract, and faecal samples to collect bacterial DNA from the gut, along with blood serum from turkeys at six different ages between hatching and sixteen weeks. Using sophisticated DNA sequencing technologies, they were able to identify bacterial strains from these sample by their unique genetic markers. They found that communities changed dramatically early in life, with increasing bacterial diversity apparent from hatching to five weeks. However, by eight weeks of age, these bacterial communities had stabilised and remained consistent through the final sampling at sixteen weeks of age. Bacterial communities in the gut and sinuses showed the most stable compositions, while tracheal communities tended to vary more.

Dr Lee's group is currently in the process of analysing blood serum to identify if any of the tested turkeys had been exposed to any pathogens that may have influenced their microbiome. This data provides a baseline for the commercial turkey microbiome and will be used in future work to understand how the microbiome changes in response to an infection. The team also hope to identify virus strains that may be present in the microbiome, which are often more difficult to collect than bacterial samples, due to their delicate nature.

The Fight Against the Flu

Worldwide, influenza (flu) is one of the most actively evolving virus families, with new variations affecting millions of people and animals every year. The ability of the flu virus to frequently jump between hosts – ranging from most mammals, to birds, to humans – contributes to its ability to evolve new variants and spread quickly, and increases the possibility of evolving deadly traits each season. This unique trait is why flu vaccines often need to be administered every year to stay effective, as each year actually brings new strains of the flu virus. A universal vaccination that protects against many variants of the flu and protects a wide variety of species could reduce the need for yearly vaccines, and prove transformative in worldwide efforts to prevent a flu pandemic.

Dr Lee has been studying transmission of the flu within and between species, has used different animal models to understand the pathogenesis and efficacy of different vaccine formulations, and has identified key shared molecular and immune factors that could make a universal vaccine possible. This data not only helps shape vaccines, but also informs outbreak management strategies to prevent spread across species. Dr Lee and his research team are developing new flu vaccines and vaccine formulations that could work on multiple species, and have identified the best models for testing how effective a given vaccine will be in humans.

In addition to his efforts to prevent future outbreaks of deadly flu strains, Dr Lee is working with researchers in Egypt to quell the effects of Highly Pathogenic Avian Influenza, which has devastated the country with ongoing outbreaks. His team is carefully monitoring mutations in the Highly Pathogenic Avian Influenza virus (called the H5N1 virus) that could make it resistant to vaccines, while simultaneously using the information they collect from the virus to develop a more effective vaccine. They are currently developing a vaccine that could be used in Egyptian poultry to prevent further avian spread of the deadly disease.

Working Together for a Healthier World

Effective management and containment of livestock disease outbreaks requires comprehensive research across multiple facets of disease ecology and pathogen biology, along with participation and collaboration between people at every level of livestock management. Dr Lee's research team approaches problems of animal health from numerous angles, from educating farmers in how to keep their flocks healthy to developing novel vaccines using cutting edge of molecular technology. The work of Dr Lee and his team brings new light to old questions about animal health, and their innovations protect the health of innumerable humans and animals worldwide.

Meet the researcher

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The Ohio State University

Dr Chang-Won Lee began his career by earning his DVM in 1996 from Seoul National University, College of Veterinary Medicine, Korea. His interest in avian flu led him to continue at SNU to earn a Master's degree in Microbiology in 1998. He completed his education in 2001 with a Ph.D. in Microbiology from the University of Georgia College of Veterinary Medicine, focusing his dissertation on avian infectious bronchitis. After completing a post-doctoral position at an USDA poultry research laboratory in Georgia, Dr Lee joined the faculty at the Ohio State University in 2005 where he still runs his laboratory today. Dr Lee's distinguished career has led to over 90 high impact publications in topics ranging from disease ecology to vaccine development. He is active in both research and extension related to avian diseases and health and is the director of the multi-state research project, Control of Emerging and Re-Emerging Poultry Respiratory Diseases in the United States.

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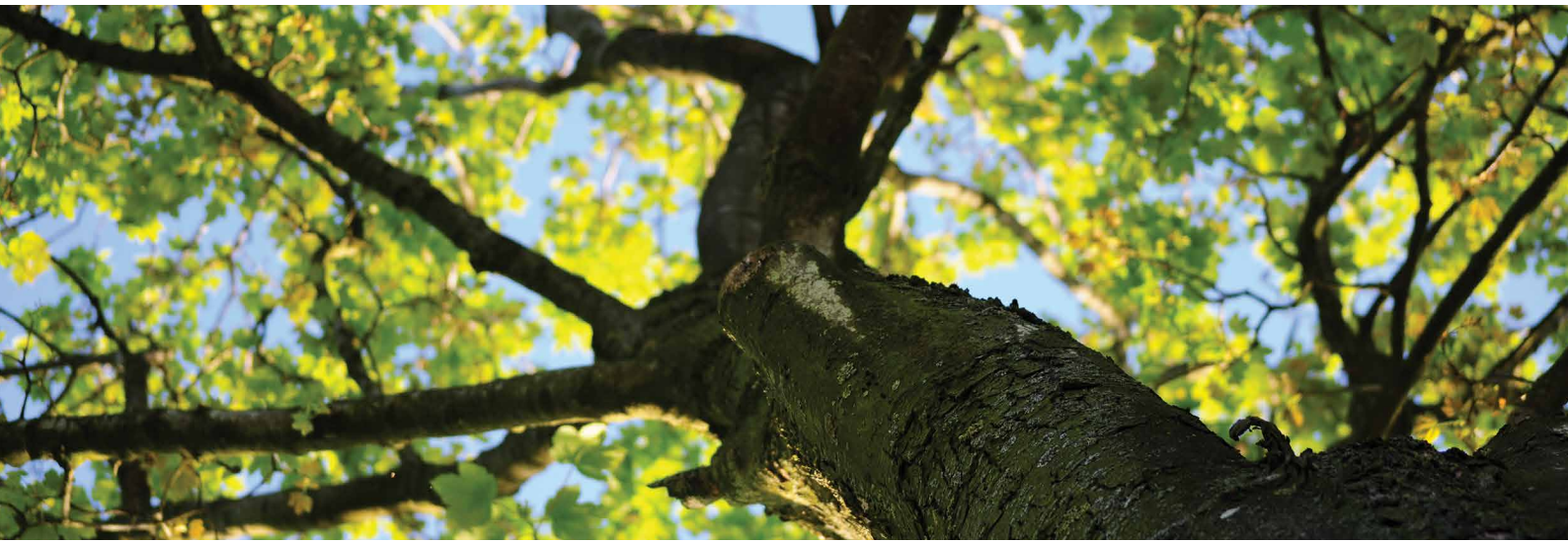
Trees for Cities

Breathing life into your neighbourhood



WHO WE ARE

Trees for Cities is the only charity working on an international scale to create greener cities. Since 1993, we have engaged over 70,000 people to plant over 650,000 urban trees in parks, streets, schools and housing estates across the UK, as well as internationally, revitalising these areas and improving the lives of the people who live in them. We strengthen communities through volunteering opportunities and inspire children to grow and eat good food and to connect with nature.

WHAT WE DO AND WHY WE DO IT

We focus on planting trees and greening community spaces where the social and environmental impact on local people is greatest. In London this might mean planting trees to clean the air or transforming unused community spaces into vibrant green areas, making our communities happier and healthier places to live, whilst in Nairobi it's planting fruit trees for food and sustainable livelihoods.

HELP US PLANT A MILLION URBAN TREES BY 2020

To date we have planted over 650,000 trees in cities. We have now set ourselves an ambitious new target to strive to plant 1 million urban trees by 2020. Help us meet this exciting new milestone...

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www.treesforcities.org/donate/

FIND OUT HOW TO GET INVOLVED AT
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MISSION

- Planting trees and greening cities worldwide.

VALUES

- **People-led:** Although our reach is global, we value the importance of a local focus. We always work through and within local communities to strengthen them and empower their members.
- **Quality-driven:** Both the quantity and quality of the trees we plant are at the forefront of our planning so that we constantly strive to maximise the impact of our projects to the environment and society.
- **Delivery-focused:** We are an organisation that gets things done. What we talk about, we do – effectively, efficiently and on-time.

WHY TREES MATTER

- Trees help our environment and the impact of climate change:
- They remove 4m tonnes of carbon from the UK atmosphere each year (Forestry Commission 2010)
- They can cool the air by 2 - 8 degrees C
- Trees absorb water , lowering stress on storm water drains and mitigating flood risk
- A single mature oak tree can host up to 423 different species of invertebrates that support birds and mammals
- Each year Trees for Cities plant around 65,000 trees in cities worldwide, revitalising cities and enhancing the lives of the people that live in them.