Scientia

REVELATIONS IN CONSERVATION & SUSTAINABLE AGRICULTURE

HIGHLIGHTS:

- Using Population Genetics to Inform Fisheries and Wildlife Conservation
- Adapting Dryland Cereal Production to Climate Change
- Utilising the Plant Microbiome in Agriculture
- The Dairy Cow: Beyond Mass Production

Issue: #117

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WELCOME...

Science Diffusion

Our ever-growing human population has led to drastic declines in Earth's biodiversity, through various factors including climate change, habitat destruction and pollution. In fact, studies have estimated that Earth is losing species at a frequency of somewhere between 100 and 1000 times the normal background extinction rate, plunging the planet into its sixth mass extinction event. Therefore, concentrating our research efforts on preserving and restoring biodiversity, while also developing more sustainable ways to feed the increasing human population, is now more vital than ever before.

In this crucially important edition of Scientia, we feature a diverse collection of promising research projects, on topics including conservation, sustainable agriculture and ethical meat and dairy production.

In our first section of this edition, we showcase the latest advances in conservation science. Here, we meet researchers who use population genetics to boost numbers of freshwater organisms, while in another article, we meet a scientist who is developing innovative ways to help trees survive and thrive in urban settings.

One of the most significant ways that humans negatively impact the planet's biodiversity is through our food production. In fact, agriculture takes up almost 40% of Earth's total land area, which has led to widespread habitat destruction worldwide. The extensive use of toxic pesticides has further exacerbated the problem, particularly for insect populations. Thus, many researchers are now developing more sustainable ways to grow food, whilst also ensuring our food security into the future. Climate change is an additional challenge that must be overcome, as our agricultural systems are extremely vulnerable to rising temperatures and extreme weather.

This is the theme of our next section, where we highlight the latest innovations in sustainable crop science – from investigating the cultivation of alternative crops that support pollinators and improve soil quality, to developing crops that are resilient in the face of climate change.

Continuing with the theme of sustainable agriculture, our last section deals with the ethical and sustainable production of meat and dairy products. Here, we showcase the work of several researchers who are developing effective solutions to our unethical treatment of livestock, from reducing the prevalence of disease amongst poultry and cattle, to investigating hunting as an alternative to intensive farming practices.

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Meet The Team...

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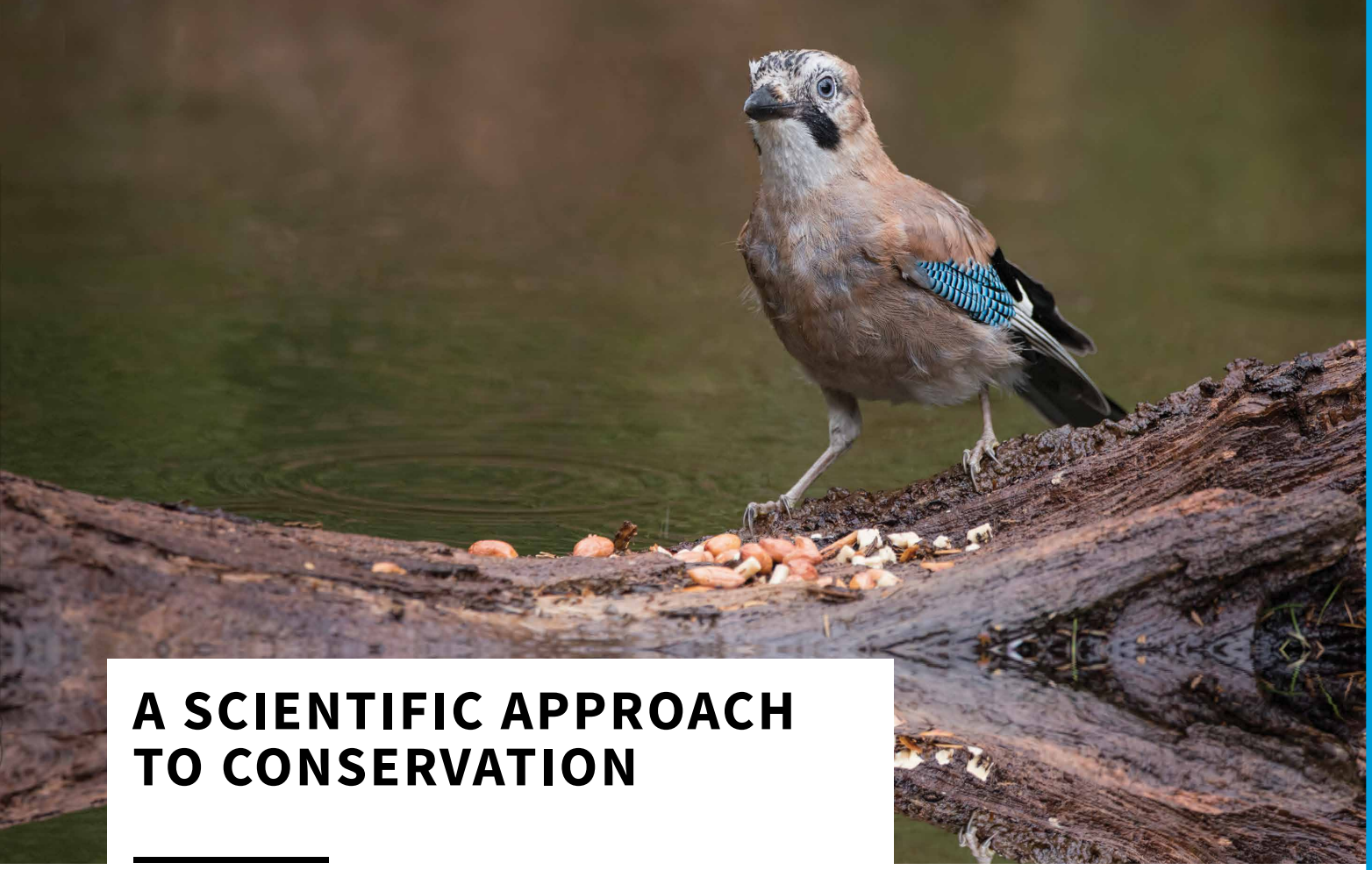


A SCIENTIFIC APPROACH TO CONSERVATION

Human activity has plunged Earth into its sixth mass extinction event – aptly termed the Anthropocene extinction. According to estimates, species are disappearing at a frequency of somewhere between 100 and 1000 times the normal background extinction rate. This disastrous decline has been caused by numerous human factors, including pollution, climate change and habitat loss to agriculture, urbanisation and deforestation.

In the past 50 years alone, scientists calculate that populations of wild vertebrates – mammals, birds, fish, reptiles and amphibians – have declined by about two-thirds. Invertebrates have taken an even greater hit. Last year, for instance, a landmark study of flying insects revealed that their numbers had plunged by a terrifying 76% in just the last 27 years. Reminding us that insects comprise around two-thirds of all life on Earth, one of the researchers involved in this study stated that we 'are on course for ecological Armageddon. If we lose the insects, then everything is going to collapse.'

Over the same period of time, the global human population has continued to grow and GDP has exploded. Consuming the planet's resources, while also using Earth as a dumping ground, are two of the major reasons behind this increase in human prosperity. Therefore, since human beings are the perpetrators behind the enormous decline in Earth's biodiversity, it is us who must take action to begin restoring it.

However, developing effective conservation strategies can be extremely challenging, and the stakes are too high for our efforts to fail. Species reintroduction programmes can often work very well, but these strategies often accidentally lead to populations that are not well suited to their once-native homes, and low genetic diversity. Such genetic diversity is essential for a species to survive on our ever-changing planet, allowing it to persist long after conservation efforts have ceased.

Therefore, a good understanding of the genetic variation across a species is essential – and this is exactly what we will explore in the first article of this section. Here, we meet Drs Eric Hallerman and Jess Jones at Virginia Tech University, who use population genetics to inform conservation management plans that protect the genetic diversity of aquatic organisms while also bolstering their populations. Their research has already helped to restore numerous fish and mussel populations in the southeast US.

Also interested in the health of freshwater organisms is Dr Deb Jaisi at the University of Delaware. In the next article of this section, we feature his team's work in investigating phosphorus pollution in waterways. Excess phosphorous in rivers and lakes primarily originates from crop fields, where it is applied as fertiliser, and can give rise to algal blooms that can have a devastating impact on aquatic ecosystems. Dr Jaisi's team has achieved deep insights into the specific mechanisms by which phosphorus leaches from agricultural land and cycles through ecosystems. Such insights will no doubt offer government bodies the information they need to design effective strategies to curb phosphorus pollution, thus helping to maintain healthy ecosystems and water quality.

From freshwater ecosystems, we move on to a heavily polluted marine ecosystem – the Sargasso Sea. Covering approximately two million square miles surrounding the island of Bermuda, this vast body of water hosts mats of floating Sargassum seaweed, which are home to very specialised communities of animals, including 11 species that are found nowhere else in the world. In this section, we introduce the Sargasso Sea Commission, an international team of researchers and policy makers who are working to preserve and protect this marine treasure.

Next, we highlight the research of Dr Mike Allen of Plymouth Marine Laboratory, who uses aquatic organisms (algae) to help solve a variety of environmental problems. In one of their many research projects, Dr Allen's team found a way to exploit harmful algal blooms to generate biofuels. In another project, the team used single-celled algae to remove heavy metals from polluted water in an abandoned tin mine.

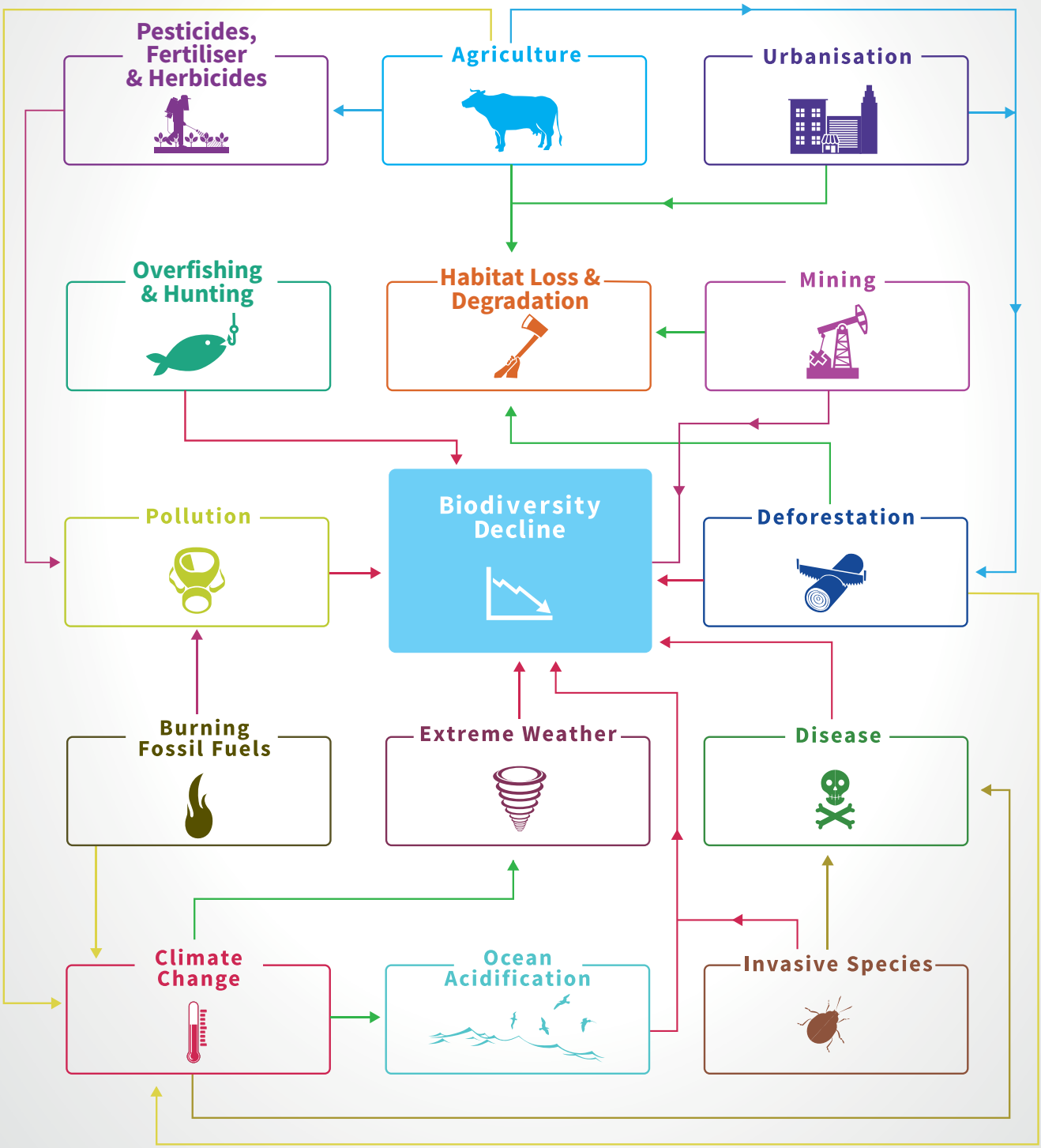
Single-celled organisms are also the focus of our next article. Here, we feature the work of Dr Rachel Mackelprang of California State University, who studies microbial communities that live deep within the permafrost in arctic and sub-arctic regions. With the permafrost rapidly thawing due to global warming, these microorganisms are becoming more active, feeding on previously frozen organic matter and releasing greenhouse gases, further exacerbating climate change. Dr Mackelprang's insights will reveal just how dangerous this feedback loop could be, which could help in the design of conservation efforts to prevent further thawing of permafrost regions.

Of course, one of the most natural methods to mop up excess greenhouse gases in the atmosphere and reduce air pollution is to plant more trees. Indeed, widespread deforestation is one of the key drivers behind climate change, as well as biodiversity loss, as trees provide habitat and shelter for a vast variety of different organisms. Here's where the work of Dr Nina Bassuk at Cornell University comes in. In the next article, we introduce Dr Bassuk and her colleagues, who have been developing ways to help trees survive and thrive in urban environments.

Also advancing the use of nature-based solutions in urban areas is Dr Benedetto Rugani and his team at the Luxembourg Institute of Science and Technology. In the next article of this section, we introduce their innovative projects, which are helping cities to leverage ecosystem services to improve residents' quality of life, reduce pollution, and boost biodiversity, creating a healthier, more sustainable planet for everyone.

And of course, our conservation section would not be complete without focussing on one of the most threatened group of animals on the planet – insects. As previously mentioned, the abundance of flying insects has dramatically dropped by a terrifying 75% over the past 27 years. Because insects are so important to life on Earth, acting both as pollinators for plants and prey for other animals, many scientists are attempting to find ways to maintain and restore their populations. In the final article of this section, we meet Dr Timothy Schowalter at Louisiana State University, who has been studying insect communities in forests for decades. Dr Schowalter suggests that by working with insects in an ecosystem approach, we could enjoy healthier environments for years to come.

FACTORS BEHIND BIODIVERSITY LOSS



USING POPULATION GENETICS TO INFORM FISHERIES AND WILDLIFE CONSERVATION

Conservation can be difficult work on an ever-changing planet, where human activity often runs counter to the needs of endangered species. When budgets and resources are limited, it is critical that conservation plans are designed to be as effective as possible. **Dr Eric Hallerman** and **Dr Jess Jones** use population genetics to help conservationists develop management plans that protect the genetic diversity of aquatic organisms while bolstering their populations.

Worldwide, the effects of climate change and habitat loss have led to declines in the populations of many organisms, and every year an increasing number of plants and animals appear on the International Union for Conservation of Nature's Red List for imperilled species. This is particularly true of aquatic life in the southeast United States, where many freshwater and marine animals are rapidly declining due to factors such as overfishing, agricultural runoff and industrial pollution, dams, and the withdrawal of water for human activities.

However, designing effective conservation programs can be complicated work, particularly for species whose natural population structure is poorly understood. While habitat restoration and species reintroduction can be good strategies, it is the genetic diversity of a species that helps it to survive in a changing world, and makes it most likely to persist once conservation efforts have ceased. Without a good understanding of the genetic variation present across a species, where some groups might be specially adapted with unique traits, it is easy for well-intended efforts to accidentally lead to inbred populations at high risk for disease or less suited to their once-native homes.

Dr Eric Hallerman of Virginia Tech University and Dr Jess Jones of the US Fish and Wildlife Service, who is stationed at Virginia Tech, apply the tools of population genetics to conservation biology to help save aquatic animals in the southeast US. Their research has helped to restore numerous fish and mussel populations and provided frameworks for others to follow when planning effective conservation efforts.

Keeping it Local

Among the greatest difficulties when embarking upon a conservation effort is understanding the best way to manage the remaining natural populations. Often it is assumed that all the members of a species are similar enough to be treated the same and even interbred with little consequence, but in reality, this is seldom the case.

Species are often divided into populations – distinct groups of the same species that live in the same area, experience similar challenges, and primarily interact with and mate with one another. While members of different populations are still the same species, and can produce offspring together if they meet by chance, often the different conditions that they experience lead to minor changes in their genetics and fitness-related traits, known as 'local adaptation'. When members of a locally adapted population are transplanted to the conditions experienced by a different population, they may not fare as well.

Conservation efforts that fail to recognise local variation between populations may fall flat. However, this variation can be difficult to detect by observation alone, particularly

for small aquatic creatures such as freshwater mussels. Fortunately, modern genetics tools offer a window into local adaptation that can help researchers parse out which populations might be unique.

Drs Jones and Hallerman have developed the use of genetic data to inform conservation decisions into an art. Using modern molecular genetics techniques, they routinely identify where the variation in populations lies, and use this information to advise conservation agencies in how to manage species to both protect the uniqueness of locally adapted populations and preserve genetic variation across the species.

The habitat of a threatened Virginia fish, the Roanoke logperch, has been heavily fragmented by the damming of its native rivers, creating a patchwork of dam-divided populations across the region. In 2013, Dr Hallerman's lab completed a comprehensive survey of the current populations of the fish, in an effort to determine how their numbers could be restored. They found that the fragmentation had led to a loss of genetic variation in certain areas, and recommended that to restore the Roanoke logperch, it would be necessary to reconnect certain isolated populations.

Similarly, a research team led by Dr Jones set out to characterise the genetic structure of remaining populations of the endangered rough pigtoe mussel, found in rivers in Tennessee and Kentucky. First, they determined that the rough pigtoe is indeed a unique species, distinct from its close relatives. Then they found that populations from the two states are similar enough to be considered a single species. Although they are the same species, the two populations are genetically distinct, and Dr Jones recommended that they should be managed as distinct units.

Though not imperilled, the horseshoe crab is of high ecological and economic importance to the Eastern seaboard, due to its unusual copper-based blood which is harvested for medical applications. However, habitat loss and harvesting have led to a steady decline in horseshoe crab populations. Recently, Dr Hallerman's team sampled crabs along the shore from Maine to Mexico, and found that there are regional differences between populations. This supports the formation of a region-based management system that can maintain demographically viable crab populations.

Drawing Lines Between Species

In addition to characterising known species, Dr Hallerman and Dr Jones often find evidence for new species in their work.

Mussel species can be particularly difficult to distinguish by assessing their shells alone. During his work on the highly endangered *Epioblasma* genus of mussels, they found that one group of the small creatures was indeed a unique species, while another with a slightly different shell qualified as a subspecies.

Another group of critically endangered mussels, the Cumberland bean and Tennessee bean, have highly similar shells but with different colours – white and pinkish purple on the inside, respectively. It was

believed that the two overlapped in habitat, making management decisions for their conservation difficult. When the team investigated, they instead found that the two are not only distinct species, but that their ranges do not overlap at all. Rather, white-shelled mussels that had previously been identified as Cumberland beans, were actually just another colour variation of the Tennessee bean mussel. Although the white shells of the two species are not distinguishable by eye, their genetics tell another story.

The Hatchery Problem

One of the most common means of conservation intervention for endangered aquatic animals is hatchery-based rearing with subsequent release into the wild. While this may seem like a straightforward enough task, without a careful program design that helps preserve genetic variation, hatcheries are at risk of negatively altering the genetic makeup of the populations they are trying to preserve.

This most commonly happens through unintentional artificial selection. Artificial selection occurs when only plants or animals with a particular trait are allowed to breed – this is the method by which most of our modern crops and domesticated animals have come to exist in the forms we recognise. Pure-bred dogs are among the most familiar examples – there is only one species of dog, but through breeding for a specific trait, such as short legs in the dachshund, humans have created a myriad of dog breeds that look nothing like their wolf ancestors.

When artificial selection occurs in hatcheries, it is often less obvious, and is more likely to be due to the conditions in the hatchery itself or the conditions under which the breeders for the hatchery were collected. In the first case, conditions in the hatchery may be different than those in the wild and cause young animals sensitive to those conditions to survive at lower rates.

For example, imagine that the water temperature in a hatchery is a few degrees cooler than what animals would normally experience in their native stream. Very young offspring that are cold-hardy would be likely to survive to adulthood, while those that need warmer waters die off. However, when returned to their natural habitat, the individuals that thrived in the hatchery may struggle to deal with warmer water.

In the second case, many animals breed over the course of a season, with some individuals that preferentially breed early and those that preferentially breed late. If well-intending conservationists only collect breeders during one time point of the season, they are likely to exclude genetic variants unique to early and late breeders.

In both of these situations, artificial selection has occurred, which may impede the success of any reintroduction programs.

Innovation in Hatchery Conservation

Dr Jones and Dr Hallerman have made strides in improving hatchery programs for both mussels and fish, and helped others prevent common errors that put hatchery conservation at risk of failure. As population geneticists, their chief goal is to build restocking programs that maintain the genetic diversity of the original population.

As part of his extensive research in captive mussel propagation, Dr Jones has developed a comprehensive set of guidelines that can inform other programs. He posits that first and foremost, there should be a habitat to introduce the animals back into. Next, it should be determined what the current genetic variability of a target population is, and what the plans are for collecting and maintaining this genetic diversity. Throughout the entire hatchery rearing process, Dr Jones's team is initiating a program to ensure that genetically diverse progeny are stocked into the wild.

It is critical for conservationists to be aware of subpopulations that may have their own genetic structure and may have unique traits that would be diluted or lost by interbreeding with larger populations. Breeders should be collected from the same areas that their progeny will be returned to, ideally during multiple points in the season and periodically rotating out breeding adults. Hatchery habitats should mimic natural ones as closely as possible, providing variation in temperature and other factors that offspring will face when returned to the wild. And finally, release programs should be carefully designed and monitored to identify which factors facilitate the most successful boosts to wild populations.

Following these guidelines, Dr Jones and Dr Hallerman were able to lead a program in Virginia that successfully bolstered the population of endangered oyster mussels and identified the best release methods for this particular species.

These methods have also helped to bolster the populations of sport fisheries. The walleye is a popular and economically important sport fish in the eastern US, but the population of these fish crashed after hydroelectric dams were constructed in the New River of Virginia. Initial stocking programs introduced fish from other locations, but this practice was suspended in 1997 when it was realised that this process conflicted with the conservation of native walleye.

Through genetic testing, Dr Hallerman and his colleagues were able to first identify native individuals, and then develop a breeding program to focus on restoring their numbers. Over 17 years, the team was able to successfully restore the population to historical numbers, while simultaneously increasing the frequency of native walleye. By marrying genetics and conservation, Dr Hallerman's team has been able to ensure that these fish will retain their natural variation while remaining available for sport fishing.

A Future for Aquatic Conservation

As more aquatic species face endangered status and budgets tighten, it is becoming ever more critical to identify the best approaches to population management. Through the work of Dr Hallerman and Dr Jones, fisheries managers now have a better understanding of how to plan and implement cost-effective conservation strategies that are proven to work.

Meet the researchers

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Dr Eric Hallerman is a professor in applied population genetics and biotechnology. His research interests include genetics of fish and wildlife species, genetic improvement of aquaculture stocks, animal biotechnology and related public policy, and genetics education. He completed his BS in environmental science in 1977 and his MS in biology in 1980 at the University of Illinois at Champaign-Urbana, followed by a PhD in fisheries and aquaculture at Auburn University in 1984. He then worked as a post-doctoral fellow at Hebrew University of Jerusalem, and as a research scientist at the University of Minnesota. He joined the faculty at Virginia Tech in 1989, where he maintains an active molecular genetics laboratory. He was also head of the department between 2007 and 2013.

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Dr Jess W. Jones is a restoration biologist with the US Fish & Wildlife Service and director of the Freshwater Mollusk Conservation Center. He is also an associate professor in the Department of Fish and Wildlife Conservation at Virginia Tech. His research focuses on conservation biology of freshwater mussels, with an emphasis on: conservation aquaculture; restoration ecology and monitoring of populations; aquatic ecotoxicology; and conservation genetics. He is particularly interested in improving field and laboratory techniques to restore mussel populations to enhance the capability of the Department of Interior's Natural Resource and Damage Assessment and Restoration (NRDAR) program. This work includes applying small- and large-scale mesocosms to improve captive growing and experimental conditions for endangered mussels and fishes. He earned his BS in 1996, his MS in 2004 and a PhD in 2009, all in fisheries science at Virginia Polytechnic Institute and State University.

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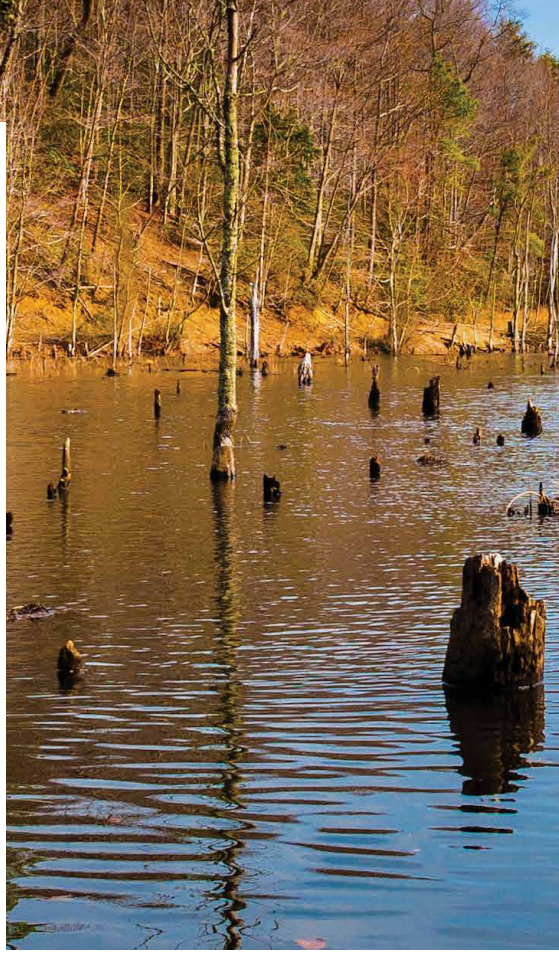

IN SEARCH OF A NEW PHOSPHORUS-CYCLING PARADIGM

Phosphorus poses a modern-day dilemma. On one hand, it is an essential nutrient for all plants and animals and is undisputedly indispensable as a crop fertiliser. On the other hand, its wide use in agriculture has been blamed for polluting waterways. Phosphorus is also becoming scarcer, so clearly a new approach is needed for its management. **Associate Professor Deb Jaisi and his team** at the University of Delaware are developing novel ways to track phosphorus as it moves and transforms through the environment. Their findings are already changing some long-held assumptions.

The Peculiarity of Phosphorus

Phosphorus is an essential element. It's found in our bones and is a key component of adenosine triphosphate, or ATP, our cells' energy source. We acquire phosphorus by eating plants or other animals that eat plants, which absorb phosphorus from the soil. Phosphorus in the soil either originates from the decomposition of plants and animals, the weathering of rocks, or is externally applied for plant growth.

In today's world, however, we mostly eat plants, or crops, that have been grown in intensely-farmed fields. Crops deplete the soil of so much natural phosphorus that it has to be mined from elsewhere and added to the soil as fertiliser. This is where our modern-day problem with phosphorus begins.

As it is, humans are mining and moving phosphorus in ways that are interrupting its natural cycle. 'The world today is a more challenging place than before in many dimensions – one being the disproportionate distribution of phosphorus,' says Dr Deb Jaisi of the University of Delaware. Dr Jaisi and his research team are keenly interested in how phosphorus transforms and transfer from soils to waters, especially given the problems it poses.

One problem is that phosphorus (or P) is that it is a non-renewable resource. It's mined from phosphate rock, which is only found in a limited number of reserves. Morocco and the Western Sahara account for around 75% of the world's total phosphate reserves, while Florida in the United States accounts for most of the rest. The limited availability of this resource means that factors such as political instability can disrupt the global supply at any time.

There is no substitute for phosphorus, especially in its important use as a fertiliser. 'The natural reserves of phosphorus are alarmingly declining and the supply of fertilisers for future crop production has been an even more pressing question,' expresses Dr Jaisi.

Along with its growing scarcity, another problem is that phosphorus is a major source of water pollution. Plants need a high amount of phosphorus to grow, but if a small amount of phosphorus is lost to open waters, that is sufficient to cause water quality problems. This is because a limited amount of dissolved phosphorus can significantly boost the growth of algae and aquatic plants in surface waters, which in turn depletes oxygen and causes larger organisms such as fish to die in deeper waters. These undesirable conditions, known as 'eutrophication' at the surface water and 'dead-zone' in bottom waters, have always been blamed on excess phosphorus and nitrogen originating primarily from agricultural lands. However other sources such as sewage and geological sources could also be a source of phosphorus pollution.

The issues of dead zones and eutrophication, especially in waterways such as the Chesapeake Bay and the Gulf of Mexico, have been mulled about for decades. 'These problems have attracted media attention for decades and are still continually highlighted in news stories and reports to highly significant scientific bodies such as US National Academy of Sciences and policy bodies such as scientific report to the White House,' says Dr Jaisi. However, he thinks that the old ways and methods of looking at the problem aren't going to generate useful results that may lead to effective strategies for cleaner waterways.

‘Our findings will make meaningful contributions to the goal of maintaining healthy ecosystems and water quality, while at the same time achieving sustainable agricultural production – which is central to meeting the water, energy, and food security demands of a growing global population.’

‘Ironically, classical methods used in phosphorus research cannot resolve these questions,’ he says. Dr Jaisi explains that we instead need to move onto new analytical methods that are specific and can identify and connect phosphorus sources and processes.

Dr Jaisi has made understanding phosphorus the goal and focus of his research ever since he became aware of the environmental problem it poses. ‘I am passionate about breaking the technological barriers and develop and apply novel methods – that bring benefit both to science and society,’ he says.

Environmental Biogeochemistry Laboratory

Dr Jaisi pursues his passion and leads the Environmental Biogeochemistry Laboratory (EBL), located in Newark, Delaware. EBL has both an analytical biogeochemistry and a stable isotope geochemistry facility, the latter boasts state-of-the-art instruments and equipment. Since 2011, Dr Jaisi and his team have received $5.5 million in funding from a mix of federal funding bodies (USDA, NSF and USGS), professional organisations, and private sources.

The team uses stable isotopes fingerprint to trace and mark phosphorous as it is added to soils and is then lost to the ground and surface water. They also use isotopes to distinguish between different generations of so-called legacy phosphorus. Legacy phosphorous refers to the build-up of phosphorous in soil over time due to the constant application of manure and fertiliser in excess of plant needs. As of now, available research tools to discriminate legacy phosphorous and address the issue of their fate are essentially null. This means that the role of legacy phosphorus is approximated.

Dr Jaisi is keenly interested in the fate of organic phosphorus compounds, as some of them are highly potent for water quality issues and others are present in pesticides. For example, phytate is a naturally-occurring organic phosphorus compound found in plant seeds and can account for a high amount (40–55%) of the total phosphorous in fertilised agricultural soils. Contrary to the ‘common wisdom’ that phytate and its daughter products are relatively inert, Dr Jaisi’s team found that it is much more reactive than previously thought and are in the forefront on developing methods to track these chemicals in the environment.

Dr Jaisi recently received an NSF CAREER award to undertake research into the fate of phytate in the environment. Another compound of interest for research is glyphosate, a widely used herbicide (known as Roundup to almost every gardener in the US or UK). On the plus side, glyphosate is effective and inexpensive. However, its toxicity and mobility in the environment are questioned by the public and policy makers. Dr Jaisi and his collaborators are using advanced research tools, including stable isotopes and computational modelling, to identify the sources of this herbicide and its pathway of degradation in the environment. Dr Jaisi believes that the mechanistic understanding of degradation recently generated may open up possibilities to ‘bias’ the degradation to safer product pathways or could be used to design safer herbicides.

The Chesapeake Bay Studies

Dr Jaisi’s most important field site – the Chesapeake Bay – is literally in his back yard. It is the US’s biggest and most productive estuary that is also located in a densely populated and politically sensitive area – next to Washington DC. As such, it has suffered years of runoff and pollution from agriculture, farming, industry and general urbanisation. Eutrophication issues and dead zones abound and very little achievement in water quality achieved so far in the Chesapeake.

For the last few years, Dr Jaisi and his team have set out to map and better understand phosphorous-cycling in the Chesapeake

Bay – both recycling within the Bay and incoming from land sources through rivers. Using their novel methods with stable isotopes, they are shedding new light on where phosphorous comes from and how it impacts water quality.

One of their studies, published in 2015 in the *Soil Science Society of America Journal*, focused on East Creek, a tributary on the eastern shore of the Chesapeake Bay. This creek drains runoff primarily from agricultural fields and poultry operations for years, causing it to have a significant build-up of soil and legacy phosphorous. Their study identified the major factors that influence how much phosphorous is actually mobilised downstream into the Chesapeake Bay. These results were highlighted in the CSA news magazine. In follow up studies on this creek, the team has evaluated the roles of the tide on diluting and removing phosphorous from the upstream region of the creek. Similarly, combining isotope signatures with element fingerprinting has allowed the team to understand source and function of phosphorus regarding its impact on water quality.

A major research focus of Dr Jaisi's team involves the Chesapeake Bay itself. For the first time ever, these scientists demonstrated that the decay of organic matter – a process known as remineralisation – was the predominant pathway of phosphorous-cycling in the Chesapeake Bay. They were able to trace the isotopic signature of phosphorous after remineralisation and discriminate this from external phosphorus sources that are exported to the Bay, providing strong evidence on the role of internal cycling on water quality, something that has also never been previously reported. This finding was highlighted in 2015 in the cover page of the journal *Environmental Science & Technology* and several other news outlets including UDaily news and US DOE National Laboratory.

Most recently, Dr Jaisi's team has launched a multi-year study that involved collecting water and suspended particles at several sites in the Chesapeake Bay and in the Susquehanna River. The sites are meant to represent external input sources, gradients of river estuaries and freshwater versus brackish waters. By connecting phosphorus sources and cycling, the team will attempt to link summertime eutrophication with hypoxia in the main channel.

As part of this study, Dr Jaisi and his colleagues have been able to demonstrate again that remineralisation is a significant source of dissolved phosphorous in the water column in the Chesapeake Bay. They found that more phosphorous comes from the remineralisation of suspended organic matter, such as phytoplankton. Interestingly, they discovered that iron-bound phosphorous is more inert and largely deposited in the sediment column, while preserving its land-source isotope signatures. By looking at phosphate oxygen isotope ratios, the team was able to identify both terrestrial input and organic remineralisation as major sources of phosphorous in the Bay. These findings were published in 2017 in AGU's *Journal of Geophysical Research*.

A Better Toolbox

Dr Jaisi's findings on the ways in which phosphorous cycles in the Chesapeake Bay are shaking up paradigms. For years, the eventual fate of phosphorus leaching from agricultural fields and other sources, and its impacts on water quality, have remained and are hotly debated by the public and policy makers. Understanding how particular sources of phosphorus or particular processes are responsible for causing eutrophication and dead zones in the Chesapeake Bay is an area where Dr Jaisi's team has made significant contributions.

'Our work, in various dimensions from the molecular to ecosystem levels, should have profound implications for how to control nutrient over-loading in the future, especially given the high price tags associated with clean-up today,' says Dr Jaisi. 'What is important to look carefully is the cost of improving water quality. For example, economic benefits provided by the Chesapeake Bay watershed are calculated to be ~$130 billion annually *if* the Chesapeake Clean Water Blueprint is fully implemented but this comes with a hefty price tag of $5 billion annual expenditure for its clean-up.'

The team will continue to hone and tweak their procedures as they study how phosphorous behaves. 'We are using, revising and developing the toolbox of advanced and innovative analytical methods which go beyond operationally defined phosphorous pools and are able to access phosphorous structural information and then connect structure to chemical reactivity, sources, and pathway of transformation,' Dr Jaisi explains.

With good reason, he adds, 'our findings will make meaningful contributions to the goal of maintaining healthy ecosystems and water quality, while at the same time achieving sustainable agricultural production – which is central to meeting the water, energy, and food security demands of a growing global population.'

Meet the researcher

Dr Deb P. Jaisi
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Dr Deb Jaisi is currently Associate Professor of Environmental Biogeochemistry in the Department of Plant and Soil Sciences at the University of Delaware. Much of his academic career relies on training and research experience in isotope geochemistry at Yale University. At the University of Delaware, Dr Jaisi heads the Environmental Biogeochemistry Laboratory (EBL), which focuses on phosphorous cycling in both soils and waterways. EBL is home to state-of-the-art facilities for measuring and analysing biogeochemical and isotopic processes from the nano- to ecosystem- levels. Since 2011, he received about 5.5 million US dollars in funding for his research programs. Most notably, Dr Jaisi received the National Science Foundation's CAREER award in 2016. He is a member of the American Geophysical Union and the American Chemical Society, among other professional organisations. He is an associate editor of Clays and Clay Minerals and editorial board member of two journals, *ACS Earth and Space Chemistry* and *Soil Methods.* He has published over 52 peer-reviewed papers and book chapters and has given several keynote presentations in professional society meetings, state and local government bodies.

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THE SARGASSO SEA COMMISSION: SAVING THE ATLANTIC GOLDEN RAINFOREST

The Sargasso Sea contains a unique ecosystem based upon floating seaweed. It is a marine treasure that is threatened by pollution and other human activities. **The Sargasso Sea Commission,** an international team of researchers and policy makers, is working to preserve and protect this wonder of the ocean.

The Golden Rainforest of the Ocean

In the North Atlantic Ocean, surrounded by clockwise rotating currents, lies a refuge of calm, sapphire blue water renowned for its exceptional clarity and unique biodiversity. The Sargasso Sea is an unusual gem in the open ocean, where golden forests of seaweed crown deep blue waters that are often crystal clear to unusual depths. Covering approximately two million square miles surrounding the island of Bermuda and part of the infamous Bermuda Triangle, it has no land boundaries but is enclosed by four major ocean currents – the Gulf Stream, the North Atlantic Current, the Canary Current and the North Equatorial and Antilles Current – the whole system forming the subtropical North Atlantic gyre.

Home to countless legends of wrecked ships and mysterious events, along with unique communities of marine life, the Sargasso Sea is an irreplaceable natural wonder. Despite its intrigue, these waters and their inhabitants are threatened by pollution, and many are working to save this amazing ecosystem.

Two species of *Sargassum* weed float on the surface of the ocean, forming clumps and rafts. Dubbed the 'golden rainforest of the Atlantic Ocean', these rafts of *Sargassum* weed are home to very specialised communities of animals. The two species that make up the rafts are the only large seaweeds that spend their entire life floating in the ocean, reproducing solely by growing new rafts from fragments that break off larger mats. With its long evolutionary history, these mats of *Sargassum* have become home to very specialised communities of animals, including 11 endemic species that are found nowhere else in the world. Many species have become adapted to living amongst the weed, including the *Sargassum* angler fish, which is camouflaged but also has modified fins to grasp the weed fronds.

The Sargasso Sea and neighbouring Gulf of Mexico are the primary areas where rafts of *Sargassum* are found. Recently, however, large quantities of the weed have become stranded on the beaches in the Caribbean and West Africa. This *Sargassum* originates in the ocean to the south of the Sargasso Sea, where the weed is believed to have bloomed rapidly due to increased temperatures and fertilisers released from the Orinoco and Amazon rivers. The resulting blooms are carried into the Caribbean by ocean currents.

Beyond the creatures that live exclusively in the seaweed rafts, many species of endangered sea turtles hide in the *Sargassum* rafts during their

CREDIT: JP Rouja


CREDIT: Andrew Stevenson

formative years to escape predators, eat, and grow big enough to brave the open ocean as adults. The larvae of several fish species are often found associated with *Sargassum* and numbers of fish species use the Sargasso Sea as a breeding ground and migratory route, including sharks, tuna and billfish. The deep water of the Sargasso Sea, below the *Sargassum* mats, is the only known spawning ground for the endangered American and European eels.

As well as serving as a habitat for a multitude of species, the Sargasso Sea's golden rainforest is highly important to the residents of Bermuda for many reasons. The golden *Sargassum* seaweed is carried inshore during the winter months, where it acts as an important food source for reef fish when it sinks to the coral reef below. In addition to providing nutrients to corals and other benthic organisms, it also provides food and habitat for intertidal organisms, which in turn are the prey of many shorebirds.

In addition to feeding the Bermuda food chain, the decomposing washed up *Sargassum* helps fortify the beaches, preventing erosion, and is used as fertiliser by island farmers. Fishing from the shores of the islands is partly sustained by the transport of fish larvae and adults that raft within the floating seaweed. The Sea is an integral part of the island chain's maritime history, as well as an important component of its economy and the economies of other bordering nations.

However, the Sargasso Sea is currently threatened by climate change and more directly by pollution and other human activities including overfishing. Any pollutants that get into the central Sargasso Sea are trapped there by the encircling currents, and the area is now home to the North Atlantic Garbage Patch – an accumulation of mostly degraded plastic covering an area larger than the state of Texas, which is held in place by the gyre.

Protecting a Natural Treasure

Among the greatest challenges facing this area are legal ones – the ocean belongs to no country. Over 50% of the planet's surface is covered by marine waters that fall outside of the governance of nations. These so-called 'High Seas' require extended international cooperation and collaboration, since they fall outside of any country's legal jurisdiction.

In 2011, the Sargasso Sea Alliance – a collaboration led by the Government of Bermuda – began laying the groundwork for international collaboration that could provide stewardship for the area. Three years later, the Hamilton Declaration and Sargasso Sea Commission were borne out of those efforts, in the process developing a conceptual framework for how international waters could be collaboratively managed.

In 2014, the Hamilton Declaration on Collaboration for the Conservation of the Sargasso Sea was signed, recognising the ecological, economic, and research value of these waters. The Declaration was initially signed by five governments – Bermuda, the Azores, Monaco, the United Kingdom, and the United States. The British Virgin Islands, the Bahamas, and Canada joined in 2016, followed by the Cayman Islands in 2017. Other governments are considering signing also.

The Declaration establishes stewardship over the Sargasso Sea and aims to encourage and facilitate research into the wildlife and effects of pollution on the blue waters. The Declaration led to the appointment of the Sargasso Sea Commission by the Government

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of Bermuda. The Commission is a group of 'distinguished scientists and other persons of international repute committed to the conservation of high seas ecosystems', and in particular, this natural wonder. Commissioners are appointed by the Government of Bermuda after a consultation process involving the signatory governments.

'The role of the Commission members … is to exercise a stewardship role for the Sargasso Sea and keep its health, productivity and resilience under continual review,' says Dr David Freestone, Executive Secretary of the Sargasso Sea Commission. Rather than being associated with any one country, the Commission operates as a stand-alone entity, established under Bermudian law.

The Commission's Work So Far

In only a few short years, the Sargasso Sea project has set a new paradigm for conservation of areas beyond national jurisdiction, bringing together stakeholders from multiple countries and organisations. In 2011 the Alliance published a baseline study on the Protection and Management of the Sargasso Sea and the following year, the parties to the Convention on Biological Diversity, drawing on this study, agreed that the Sargasso Sea be included on a list of Ecologically and Biologically Significant Areas (EBSAs). Using the EBSA decision as a basis, special conservation measures for the Sargasso Sea were agreed upon in 2015 by the Northwest Atlantic Fisheries Organisation (NAFO) that established a moratorium on bottom trawling on Sargasso Sea seamounts in its area and gear restrictions on midwater trawling.

In 2014, the Commission brought together cable companies, marine researchers, academics, and legal scholars to discuss the environmental effects of laying submarine fibre optic telecommunications cables across the ocean floor, which provide over 97% of worldwide international communications.

The Sargasso Sea Commission is also working with more traditional conservation groups to protect specific wildlife, such as the endangered American and European eels, and collecting further data to support the need for extending protections to this incredible body of water. In collaboration with the Convention on Migratory Species, efforts are underway to define and protect the migration routes of the European eel to the Sargasso Sea as well as its spawning areas there.

The Commission has also collected data on how the *Sargassum* seaweed bolsters numerous species of fish that are commonly sought in commercial fishing, as well as charismatic and endangered sea turtles, all of which rely on the masses of golden seaweed for shelter and nutrition. Utilising this data, the Commission aims to partner with countries and commercial fishing operations to properly manage fishing activities, and ensure that these populations will enjoy the Sargasso Sea as a safe haven for generations to come. Ongoing efforts to tie fish populations to human activity are revealing even more ways that the Sea can be preserved through conservation efforts.

The Sargasso Sea Commission is also working to develop a Stewardship Plan for the Sargasso Sea – which would be the first such plan for an open high seas area. This would include parameters that could regulate different types of human activities that disrupt the golden seaweed rafts and pose a threat to the life around them. Limitations would require the cooperation of multiple countries and private sector corporations through international bodies such as the International Maritime Organization (IMO) and the Regional Fisheries Management Organizations.

A Bright Future for Blue Waters

The innovative work of the Sargasso Sea Commission is currently mainly funded through the support of private donors – it is seeking further funding to continue its important work into the future. The Commission aims to continue working to protect the Sea as a whole, while also working to protect the migratory range of the European eel, to regulate vessel activities, and to conserve threatened fishery resources in the area. It is also working with NASA to develop comprehensive satellite imagery of the area.

'We are working closely with NASA on its pilot COVERAGE project, which will provide a visual representation of its satellite data on the Sargasso Sea,' explains Dr Freestone. 'This can be overlaid with other data on animal movements and human activities in the area.' This data could help researchers identify the most critical areas for conservation efforts, and better understand how human activity affects the Sea. With the Commission's continued efforts, this beautiful natural treasure will endure for many future generations to come.

Meet the Commission

Commission as of April 2017: Wilfred Moore, Frederico Cardigos and Teresa Mackey are not pictured.

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SARGASSO SEA
COMMISSION

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Senator Wilfred P. Moore, Q.C.
Wilfred P. Moore served 20 years in the Senate of Canada and has been an outspoken advocate for policies protecting the oceans. He was a strong advocate of the Sargasso Sea Alliance, and was instrumental in persuading Canada to become the eighth Signatory to the Hamilton Declaration on December 3, 2016.
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Frederico Cardigos
With a background in marine biology and nature conservation, Frederico Cardigos has gone on to a career in island marine ecology, currently he serves the Region of the Azores as their representative in Brussels. He was regional director for the environment and maritime affairs and director of the Marine Park of the Azores.
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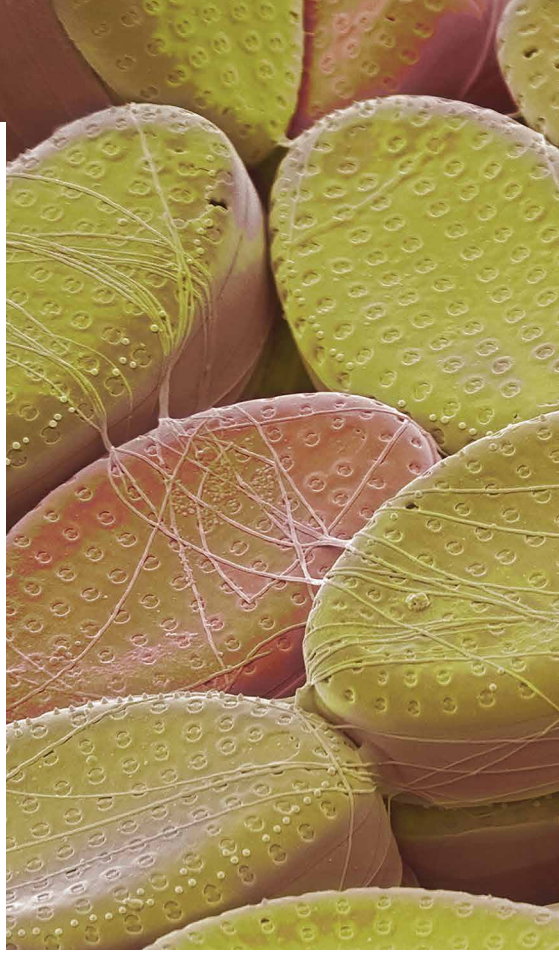
ALGAL BIOTECH: TURNING ENVIRONMENTAL PROBLEMS INTO COMMERCIAL OPPORTUNITIES



With over a decade of experience in producing solutions for industry, **Dr Mike Allen** of Plymouth Marine Laboratory (PML) is a shining star in the field of algal biotechnology. Inspired by the natural environment, he and his multidisciplinary team work to engineer and exploit algae in investigations that span the entire scale spectrum: from genetically modifying individual microscopic cells, through to producing products at pilot plant level.

Biotechnology is one of the fastest-growing sectors in the world, promising solutions to many of today's problems, such as water contamination, food production and the urgent need for fossil fuel alternatives. Solving these problems requires a multidisciplinary approach, combining the expertise of biologists, chemists, physicists and engineers, among others. Crucially, it also requires solutions that span the entire scale spectrum: from individual microscopic cells, through to pilot plant level, tackling issues relevant for industrial application.

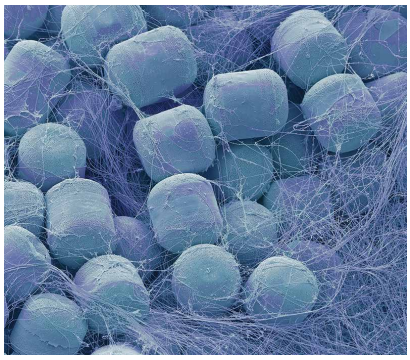
Advances in the study of single algal cells combined with genetic engineering may soon also be helping to solve environmental problems, in tandem with the generation of a range of high-value chemicals, biofuel, fertiliser and animal feed.

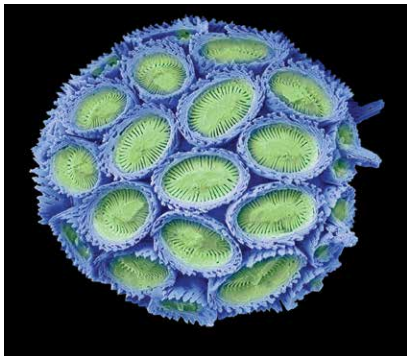
Algal Genomics

In 2016, over a decade of genomic research on microalgal coccolithoviruses (large viruses of single-cell algae) culminated in Dr Allen's team and the University of Exeter opening a state-of-the-art environmental Single Cell Genomics (eSCG) facility. Here, the genomes of individual cells and viruses can be studied without the need for usual culturing procedures. 'Working with single cells is incredibly powerful as a research tool,' Dr Allen says. 'It allows us to deconstruct complex communities and look at the individual components without the background 'noise' we usually get from mixed populations.'

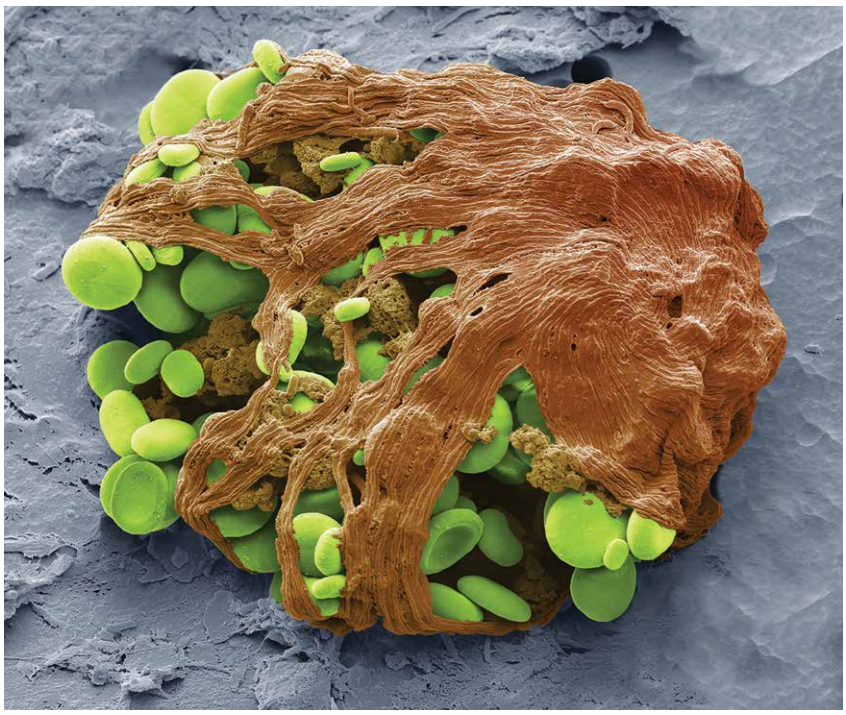
The equipment is housed within an ultraclean room, because even a single dust particle could interfere with the results. The facility houses highly-sensitive and accurate technologies, such as cell sorting equipment, liquid-handling robotics and the world's fastest atomic force microscope. Dr Allen's colleague, Dr Ben Temperton of the University of Exeter, likened it to switching from using binoculars to the Hubble telescope to study stars. It enables scientists, for example, to look at individual DNA strands on a screen. At the new facility, isolated cells of marine microorganisms are being characterised, to find out what they are doing and how they are doing it.

In a paper in the journal *Viruses* in 2017, Dr Allen and his colleagues at PML, in collaboration with scientists from the USA and Norway, compared the genomes of different coccolithoviruses. This research confirmed the idea that viruses play a central role in the transfer of genetic information among different life domains, and that viruses exert a great influence on nutrient and energy cycles in the oceans. The PML team has sequenced the genomes of dozens of algal, virus, bacterial and fungal strains in recent years. In so doing, they have begun to bio-prospect – unlocking the secrets that these genomes harbor that make them unique. For example, they identified a new intein from a virus, a useful synthetic biology tool that can be used to aid genetic manipulation, protein expression and the screening and manufacture of new pharmaceuticals.





To complement this laboratory work and aid bio-prospecting from the natural world, Dr Allen led a team to develop an improved means of sampling the marine environment using low-cost remotely operated vehicles. The innovative method preserves natural environmental mRNA *in situ*, thereby better conserving the transcriptional profile so that it can be more accurately characterised back in the lab. 'By combining cutting edge field and



laboratory analysis techniques, we lay a solid academic foundation on which we can build our biotech platforms' says Dr Allen 'It's this knowledge and understanding that provides the insight on processes that is crucial when it comes to exploitation.'

Genetically Modified Microalgae

Dr Allen's team works with several strains of Genetically Modified (GM) microalgae, with the aim to cost-effectively produce compounds of commercial relevance. These strains are developed in house, or in collaboration with key partners from the academic and commercial sectors. For instance, a species of marine diatom (a type of single-celled algae with a distinctive silicon-based cell wall) accumulates an omega-3 fatty acid called eicosapentaenoic acid (EPA) which is a valuable nutraceutical. To 'improve' the omega-3 fatty acid profile, the team, in collaboration with Rothamsted Research, exploits genetically engineered strains of the marine diatom. The researchers used GM techniques to help the strain produce both EPA and docosahexaenoic acid (DHA), which can then be used as alternative source to feed fish or even replace fish oils in dietary supplements. They concluded that their best transgenic strain ('Pt_Elo5') could become a versatile industrial platform for the production of bespoke omega-3 formulations.

The use of genetic engineering is opening up new avenues within the emerging algal biotech industry. Not just limited to producing valuable compounds, Dr Allen's team are also developing strains that are more amenable to downstream processing. 'One of the biggest issues in algal biotechnology is getting rid of all the water so that you can do something with your biomass, that's why developing downstream processing technologies is a key part of our technology portfolio,' he tells Scientia. 'GM provides an interesting option to let the biology help us with these engineering obstacles. In addition, the commercial viability of future products derived from genetically modified microalgae will require combining the production of high-value chemicals – such as β-carotene or omega-3 fatty acids – with low-value biofuels, fertilisers or animal feeds in a biorefinery approach.'

Dr Allen and his colleagues are today culturing transgenic algal strains in closed-system photobioreactors (PBRs) and contained raceway-pond systems. These systems can provide biofuels and high-value chemicals, offering a range of sustainable alternatives to non-renewable resources. In the future, genetically modified algae will need to be deployed on a large scale, potentially combining bio-remediation with a production function, with close attention being paid to environmental risk assessment in all cases. 'The potential for GM algae based solutions is enormous,' says Dr Allen. 'Despite the current unease in public opinion surrounding their use, with all the problems we're facing in the coming century, not exploiting them will simply not be an option. But we need to make sure we take a responsible approach.'

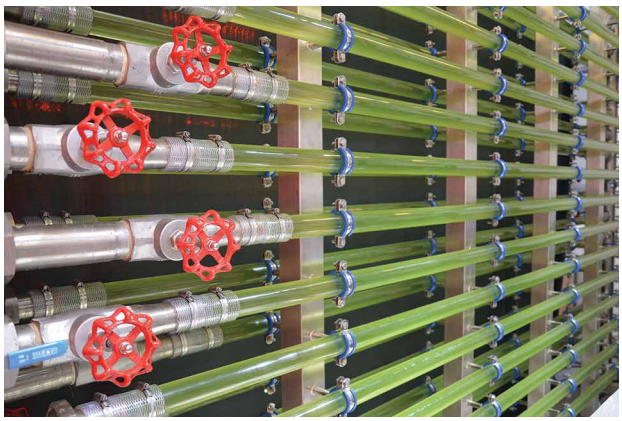
In a review article in the journal *Algal Research* in 2017, Dr Allen and two colleagues summarised the state-of-the-art for the biotechnological exploitation of GM strains of microalgae, with an emphasis on their safety with regard to the environment and human health. They concluded that the substantial knowledge base and expertise within existing aquaculture, fermentation and algal biotech industries could be combined and applied to ensure safe use of GM algae in the future.

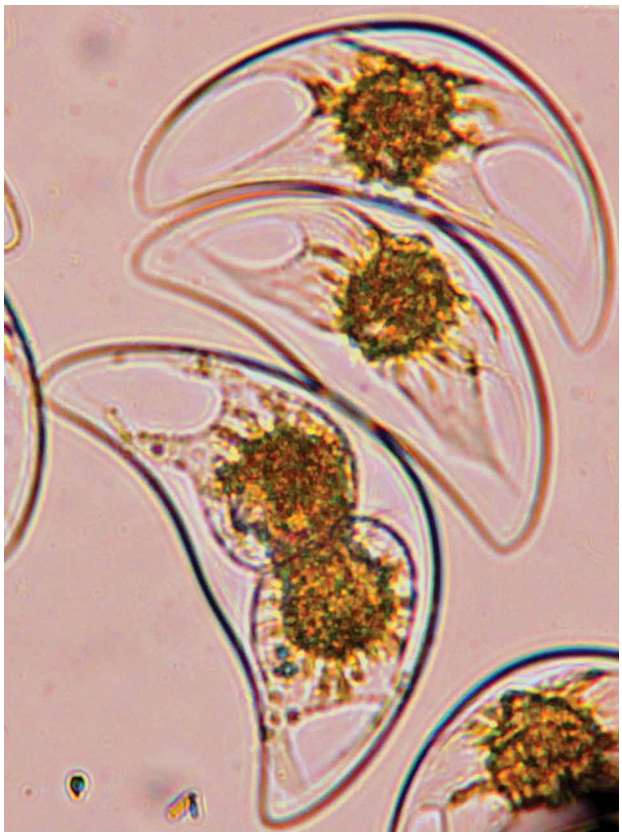
Bioremediation and Water Sanitation

Algae have considerable potential to clean up contaminated water. In 2014, Dr Mike Allen and his team at PML, in collaboration with GW4 Alliance Universities (Bath, Bristol, Cardiff and Exeter), the Coal Authority and Veolia, demonstrated the use of microalgae to recover valuable heavy metals from polluted water in an abandoned Cornish tin mine. 'Commercial opportunities are everywhere – we pride ourselves on being able to see them when they arise,' says Dr Allen. 'By making the clean-up process pay for itself, we can improve both the health and the environment of millions of people around the world.' The challenge lies in bringing the right algae into contact with the pollutants and then recovering both algae and pollutant in an energetically efficient manner. It requires matching the biology to the engineering – an approach Dr Allen's team has taken time and time again.

Sometimes the R&D path can take some unexpected routes. One technology that Dr Allen's team has pioneered is the vortex bioreactor, a system based on vortex separation systems already used by many industries. 'We aim to convert microalgae into biodiesel and harvest in a one-step, one-pot process,' he explains. 'It's ambitious, but we're making great progress. We've broken it up into a series of smaller challenges and are meeting each one.' One such challenge involved using the vortex bioreactor for membrane disruption, so that cellular lipids would become available for conversion into biodiesel. Dr Allen's team used *E.coli* for their initial trials and quickly realised they were on to something big.

Working with The Bill and Melinda Gates Foundation, the PML researchers developed the vortex bioreactor as a simple, low-cost technology for sterilising waste water.





'What started out as a technology to help convert microalgae into biofuels, spun off (quite literally) in the direction of global waste water sanitation,' comments Dr Allen. The technology exploits a continuous vortex within a pipe that brings water-borne pathogens into contact with a biocidal agent embedded in seaweed extract (in this instance copper). The team's prototype vortex bioreactors have been successfully trialled in India to TRL7, and the technology is being made freely available for humanitarian applications worldwide.

Exploiting Harmful Algal Blooms

Algae can be a major environmental problem, especially bloom-forming microalgae, which can be a source of toxins harmful to people and wildlife. Dr Allen and his colleagues are investigating methods for exploiting algal blooms, which combine environmental remediation with subsequent bioprocessing. Their approach involves utilising biological agents such as viruses, bacteria or other algae to inhibit or control bloom development, and combining this with biomass removal in order to prevent the cycle repeating. The researchers at PML envisage harvesting algal blooms as a resource, to make biofuels and other products in a cost-effective manner that could self-finance environmental clean-up operations.

Algae are known to accumulate lipids that resemble crude oil – indeed, crude oil is largely the product of fossilised algae. Dr Allen and his collaborators at Bath University have pioneered an approach for obtaining useful bio-oil from algal cells. 'The process being developed is known as hydrothermal liquefaction, which converts organic material into four phases: a bio-oil, an aqueous phase rich with phosphate and nitrate, an inert char containing stable metals, and a gas – mostly carbon dioxide,' explains Dr Allen. 'It does this using water as a solvent and through the application of high pressures and temperatures. I like to call this "geology-in-a-tin", as it mimics the natural events of millions of years ago that originally converted dead organisms into the oil we extract from wells today.'

Hydrothermal liquefaction can be applied to any type of algae. For instance, cyanobacteria (blue-green algae) accumulate several chemicals of potential commercial interest. In a study published in *Bioresource Technology* in 2016, Dr Allen's team, in collaboration with the University of Bath and the University of Natural Resources and Life Sciences in Vienna, describes how they employ hydrothermal liquefaction to convert cyanobacteria into propylene (a plastic precursor) and a bio-oil suitable for advanced biofuel production.

Turning Algae and Plastic Waste into Oil

Whilst future plastic production will be hindered by dwindling crude oil supplies, plastic waste is currently a major environmental problem. During a family walk on a litter-strewn beach, Dr Allen wondered whether the combined mess of seaweed and plastic debris could be turned into biofuel and fertiliser in one process. 'We had already managed to produce oil from algae in the laboratory and we know we can do the same with plastics,' says Dr Allen. With Dr Chris Chuck at the University of Bath, Dr Allen and his colleagues are adapting their hydrothermal liquefaction process to convert marine algae and plastic debris into bio-oil, thereby turning a problem into a commercial opportunity.

In a report in *Faraday Discussions* in 2017, in collaboration with the Vietnam Academy of Sciences and Technology, the team reported how complex and variable organic feedstocks, including algae, can best be valorised using flexible anaerobic fermentation and hydrothermal processes within an integrated biorefinery concept. They concluded that not only is there no need to separate out plastic detritus from the organic matrix prior to processing, but the plastic itself can actually improve the economic viability of the process. 'The versatile process can be applied at specific beaches, open ocean gyres accumulating debris, and at sites of harmful algal blooms,' notes Dr Allen.

Based on this approach, fast-growing algae could also be specifically cultivated to prevent excessive nutrient run off into rivers and seas (thereby preventing/mitigating HAB formation), produce oils for biofuels, high-value chemicals and other useful products (such as fertilisers for crop production) in biorefineries in a sustainable manner. To optimise the production of biofuels and other products, genetic engineering may end as a key component in the process, for example, to obtain higher yields of more valuable products without detrimentally slowing algal growth or performance.

Meet the researcher

Dr Mike Allen
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Dr Mike Allen is a Microbial Biochemist at Plymouth Marine Laboratory (PML), where he has worked since 2004. He is also an Honorary Fellow and Visiting Professor at the College of Life and Environmental Sciences at the University of Exeter and an Honorary Fellow of the School of Physics at Bristol University. After gaining his BSc from the University of Wales, Cardiff, he was awarded his PhD by Cardiff University in 2004. His 'blue skies' research focuses on understanding the role of viruses in the oceans using genomic, proteomic and other approaches. Applied projects include biofuel production and processing, water sanitation, and the development of novel bioreactor technologies for generating, harvesting and processing microalgae.

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PML | Plymouth Marine Laboratory

FROZEN IN TIME: THE PERMAFROST MICROBIOME

Deep within the permafrost, viable microbial communities persist, buried for millennia. With the permafrost rapidly thawing due to global warming, these microbes are becoming more active, feeding on previously frozen organic matter and 'breathing' out greenhouse gases, further exacerbating climate change. **Professor Rachel Mackelprang** of California State University is studying the significance of these little-understood microbes. Funded by NASA, her team is also investigating how these hardy microbes adapt and survive as an analogue of how extra-terrestrial life may survive in sub-zero space environments.

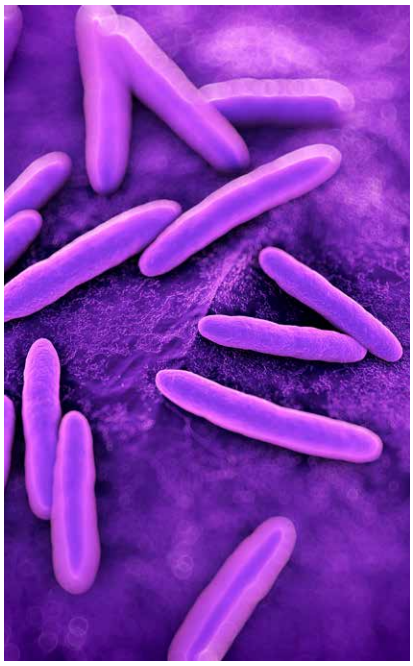
In the northernmost parts of Norway, Russia, Alaska and Canada, lie arctic and subarctic ecosystems – cold, harsh ecological habitats, with temperatures that can regularly hover near −40°C in winter. At sub-zero temperatures, the ground is permanently frozen, and is known as 'permafrost'. Permafrost may be overlaid by a layer of ice, soil or sediment that freezes and thaws with the seasons, known as the 'active layer'. Deep within the permafrost, there exist diverse communities of viable microorganisms – bacteria, archaea and even some fungi – which persist undisturbed for thousands of years. This permafrost 'microbiome' has not been extensively studied – but it's expected to play a major role in ecological events yet to unfold.

Recently, much attention has been paid to the permafrost, due to its expected significance to climate change. Greenhouse gases emitted by human activities, such as carbon dioxide and methane, trap heat from the Sun and cause changes to the climate. One worrying consequence of this is the melting of the polar ice caps, though the effects of climate change extend well beyond this. It is not just the polar ice caps under threat, but also the permafrost! The Earth's rising temperature is expected to cause large-scale thawing of permafrost – indeed this has already begun. Over the last 30 years, surface temperatures in the Arctic regions have risen by 0.6°C per decade, twice as fast as the increase in the global average temperature. Much of the permafrost is just below 0°C, meaning that even marginally small temperature increases can cause thawing, leading to the collapse of fragile permafrost ecosystems. However, the worst is yet to come.

The permafrost contains 25–50% of the world's soil carbon pool, in the form of biological matter accumulated over thousands of years. The sub-zero conditions have slowed down decay, preserving the organic matter for millennia – but the thawing set to expose this organic matter and the resulting microbial decay will release previously frozen carbon into the atmosphere as carbon dioxide and methane – further exacerbating global warming. This could set off a positive feedback loop – a chain of rapid, irreversible warming – a bad scenario for climate change.

Microbial Evolution in Permafrost Chronosequences

The existence of millennia-old viable microbial communities – literally 'frozen in time' – present unmissable opportunities for scientific study. Professor Rachel Mackelprang and her colleagues at California State University and the United States Geological Survey are studying the biology of these microbes, and their significance to climate change. 'I seek to gain a systems-level understanding of the fate of carbon in thawing permafrost



and the role microbial communities play in greenhouse gas emissions,' states Professor Mackelprang. Her team is also interested in observing how microbial communities respond to anthropogenic disturbances, and the permafrost microbes serve as excellent study models.

These so-called 'extremophile' microorganisms must contend with sub-zero temperatures, low availability of liquid water restricted to solute-rich patches of soil or brine, limited

nutrients, and high salinity. Unfortunately, at such low temperatures, the flexibility of DNA, RNA and protein molecules is greatly reduced – compromising the cells' ability to carry out even basic activities. These organisms may therefore be in a dormant phase, or be active with slowed-down metabolism and cell division.
Microbes trapped in permafrost must have counteractive adaptations for long-term survival under harsh environmental conditions. However, little is known about the survival strategies of these microbial communities that allow them to spend millennia trapped in permafrost. Professor Mackelprang and her team are trying to elucidate these microbial adaptations – at a genetic and molecular level. The team often leaves their lab in sunny California for chilly Alaska to collect permafrost samples for analysis. One of their choice locations for sample collection is the permafrost tunnel in Fox, Alaska. This tunnel, drilled by the United States Army Cold Regions and Research and Engineering Laboratory (CRREL) into a hillside, provides access to ancient permafrost from the late Pleistocene era. Beyond Alaska, they are also studying samples from global collaborators, including terrestrial and marine permafrost from Russia.

Like all microbes, permafrost microbes undergo gene (DNA) mutations and reproduce by cell division, meaning that they undergo evolution, with the harsh environmental conditions acting as 'selection pressures' that only allow the hardiest bugs to survive and multiply! A fundamental question that the research team is trying to answer is whether an increasing long-term imprisonment in frozen permafrost selects a common core set of genes, pathways, or species. A number of research groups, including Professor Mackelprang's, have found the same species and functional traits in various unrelated locations, suggesting that this is indeed the case – and a common set of functions may enhance survival under frozen conditions. For example, Professor Mackelprang and her colleagues identified the same Chloroflexi bacterial species in permafrost in three separate sites in Alaska.

Permafrost can span a number of geological epochs – including the Holocene, Pleistocene and Pliocene era. This age gradient, known as a 'chronosequence', presents a unique window into microbial evolution. Professor Mackelprang's team analyses the physical and chemical soil characteristics of chronosequences to identify the biological features of the microbial communities that are important to survival through geologic time rather than adaptations to the physiochemical conditions of the permafrost microenvironment.

Probing the Permafrost Microbiome with Metagenomics

The team uses a combination of classic microbiological techniques and cutting-edge metagenomics tools to gain insight into the evolutionary adaptations of the permafrost microbial communities. They estimate the age of the samples using radiocarbon dating, and using a battery of biochemical tests, they determine biological characteristics, including the relative abundances of dead, live and dormant cells, and indicators of metabolic activity. Amazingly, some of the millennia-old cells are successfully revived and cultured.

Most permafrost microorganisms cannot be grown in the laboratory, so the team uses DNA-based approaches such as 16S rRNA gene sequencing and metagenomics to circumvent the need for cultivation. In this strategy, DNA from the microbial community is extracted directly from the soil, and the team uses 16S rRNA gene sequencing to determine the identities of organisms living in the permafrost. RNA within the '16S' region of ribosomes (the cell's protein factories) is universally present in all bacteria and archaea. Therefore, mutations in 16S rRNA gene serve as useful markers for taxonomic identification. Metagenomics – sequencing of all DNA from the environment (not just the 16S rRNA gene) – reveals the

functional capabilities of permafrost communities. Genes encode enzymes (proteins) that drive metabolic pathways, and therefore, the information captured in the team's metagenomic analysis can identify which metabolic pathways are important for survival. Hence, these DNA-based strategies can reveal the taxonomy (species identity), phylogeny (species evolutionary history), and molecular adaptations to imprisonment in permafrost.

By applying these DNA-based tools to a Pleistocene chronosequence (19,000–33,000 years old) from the CRREL tunnel, Professor Mackelprang's group recently demonstrated the adaptive evolution that frozen microbial communities undergo. Entrapment in permafrost limits the availability of carbon, energy and nitrogen. The team found that, consequentially, metabolic pathways for utilising sugars are less abundant and instead, pathways involved in importing, breaking down and salvaging amino acids (the building blocks of proteins) are more abundant. This is because the organisms may be recycling carbon and nitrogen from proteins in detritus present in the permafrost.

Significant to climate change, Dr Mackelprang and her team also found groups of genes for 'methanogenesis' – the production of methane as a by-product of anaerobic metabolism – were frequent in microbes in the youngest permafrost. They were also found in the older age categories but were very rare. Additionally, the team found that in the older permafrost, groups of genes encoding traits that may be advantageous in ancient cryoenvironments were present at higher frequencies – these include genes for chemotaxis (the ability of single cells to move towards chemical nutrients), sensing and responding to stimuli, cold protection and dormancy. Such traits are necessary for long-term survival under cold, nutrient-deficient conditions, and it is thought that some of these genes can be transferred between organisms.

From Deep Permafrost to Deep Space

Exobiology is an exciting field of research that explores the possibility of life on other planets. If life does exist elsewhere in the Universe – how it works and how it compares to life on Earth is a matter of lively speculation amongst scientists. It is thought that studying organisms living in extreme habitats here on Earth may be useful for envisaging alien life in the most unforgiving places in space. Six of the eight planets in our solar system – as well as their moons, asteroids and comets – are permanently frozen. Therefore, the adaptations of microbial communities persisting in nutrient-limited permafrost for millennia can act as an analogue for alien life on these frozen worlds.

Therefore, Professor Mackelprang and her team are embarking on an exciting NASA-funded project to understand how the biology of alien organisms (if they exist) helps them adapt to hostile environments in space. There is some evidence suggesting that metabolically active microbes are more likely to survive long-term than dormant ones – as active cells can repair damaged DNA. Although we do not know what information storage system alien microbes would have in lieu of DNA, it may be similar, with 'active' extra-terrestrial organisms better able to carry out important 'quality control'.

Future Directions

The permafrost microbiome is poised to be a major player in climate change as the permafrost thaws, and thus accelerate climate change by releasing methane and carbon dioxide. Professor Mackelprang and her team are working to further our understanding of these microbial communities, and have already gained insights into the selection of methanogenic pathways in permafrost microbes – pathways that may soon be responsible for emitting more methane into the atmosphere. A more enlightened understanding of these processes may help us to mitigate their effects on our future climate.

Beyond our own planet, the team will also continue to explore the permafrost microbiome as an analogue for organisms that may exist in sub-zero space habitats, aiding the hunt for extra-terrestrial life.

Meet the researcher

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Professor Rachel Mackelprang is an Associate Professor of Biology at California State University, Northridge. Her research interests are centred on understanding the molecular, genetic, and biochemical adaptations of microbes in extreme habitats, in particular the Arctic and sub-Arctic permafrost, using both traditional microbiology and next-generation tools such as metagenomics. She is particularly interested in how microbial communities respond to disturbances, particularly climate change. Professor Mackelprang earned a PhD in genetics at the University of Washington, Seattle in 2006, before embarking on a postdoctoral fellowship in computational biology jointly at the DOE Joint Genome Institute and the Lawrence Berkeley National Laboratory between 2007 and 2011. In 2011, she moved to her current institution, California State University, Northridge, as an Assistant Professor in Biology, before being promoted to Associate Professor in 2016.

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CSU The California State University

TREES – THE TRUE URBAN WARRIORS

Trees benefit cities in many often-overlooked ways. They not only beautify concrete backdrops, but also improve the quality of our urban lives by providing shade, reducing storm runoff, filtering air and providing homes for birds and insects. Trees face big challenges, however, growing up in cities, largely because of drought and poor soils. To help trees survive these concrete deserts, **Dr Nina Bassuk** and her colleagues at Cornell University have been evaluating trees and shrubs for their ability to adapt, including developing resilient hybrid oak trees. A parallel research track aims at remediating urban soil conditions to reduce urban tree stress.

A Concrete Desert

Life as a city tree is not easy. For one, trees rarely get off to a good start in cities, as planners often ignore them in the early stages of urban development, and stick them in after the concrete has dried and construction is finished. 'Plants are often placed in cities as afterthoughts squeezed between buildings, sidewalks, roads and parking lots, and continually buffeted by construction and urban change,' explains Dr Nina Bassuk, a horticultural scientist at Cornell University.

Dr Bassuk, who heads the Urban Horticulture Institute (UHI) at Cornell, is a tireless advocate for city trees. She has dedicated her life to searching for ways to overcome problems they face in cities.

'Trees often find themselves being on the short-end of getting the water, the nutrients, the soil biome they need,' she says. To improve their lot in life, Dr Bassuk has spent nearly four decades improving their genetic viability and growing conditions. To accomplish this, she has had to understand what was happening both above and below the city surface.

For instance, above-ground issues include amount of sunlight, wind patterns, rain shadows caused by buildings, overhead power lines, and pollution caused by run-off of de-icing salts. Concrete, asphalt and even car hood surfaces reflect heat and can cause air temperatures around trees to rise drastically, by up to 7°C. Heat makes trees lose water faster, especially in places like large car parks, where ironically, shade is most often desired.

Below ground, city soil is notoriously deficient in nutrients and highly compacted. Tree roots often don't have room to grow because there is simply not enough soil volume, and compaction continuously occurs as construction, pavement and traffic crush the soil. All of these conditions prevent trees from getting the water they need.

Drought is, in fact, a city tree's biggest challenge. For newly planted trees, their roots can't absorb water fast enough to keep up with water loss – or 'transpiration' – from their leaves. For more established trees, their roots are often too restricted to absorb enough water and their growth suffers as a result. Paving with impermeable surfaces only further lowers or cuts off water supply. As trees dry up, they begin to lose 'hydraulic conductance' – their capacity to move water – and their ability to photosynthesise. Because of this, many trees die earlier than their forest dwelling counterparts.

Not helping matters is the fact that urbanites often plant trees that come from moist and nutrient-rich forests, when they would have been better off using trees from dry steppe or rocky mountain environments. Perhaps 'concrete jungle' is a misnomer, when in reality, a city is a concrete desert.

Forceps holding hybrid oaks propagated by tissue culture

For many years, Dr Bassuk and the UHI team have been looking to improve green areas of these concrete deserts. 'Our work deals with finding adequate space and resources necessary for healthy tree growth as well as finding the best and toughest plants that can thrive in this environment,' she explains. 'We do this through research, teaching and outreach to the professions that manage urban forests.'

Creating an Ideal Urban Tree

Since 1995, Dr Bassuk and her colleagues have focussed their urban forest studies on the oak, or Quercus, species. Oaks are not easy to propagate asexually (by grafting or rooting cuttings), so the scientists have been keen to

‘We try to improve the environment for trees, within the bounds of an urban environment, as well as evaluate and utilise the best genetics that can withstand a city’s onslaught’

Hybrid oak stoolbeds during layering procedure

Students carrying out soil assessment on design and instalment site

develop new ways to grow these popular and beautiful trees.

By 2006, they had developed over 350 unique hybrids by crossing four different oak species – *Quercus bicolor, Quercus muehlenbergii, Quercus robur* and *Quercus macrocarpa* – with other resilient oaks from across the US, Mexico, Europe and Asia. ‘These trees were created by crossing hardy native trees with compatible trees from harsh environments such as deserts and coastal areas,’ explains Dr Bassuk, adding that their efforts paid off: ‘We have over 300 unique trees that show much promise in withstanding the environmental stresses found in urban and other human impacted landscapes.’

From 2009 to 2012, the researchers planted their hybrids in a field near their laboratories in Ithaca, New York. They narrowed the number of crossbreeds down to around 30 unique hybrids that consistently produced more well-rooted shoots and were more tolerant to the cold and alkaline soils common in urban landscapes.

In a two-year study that began in 2015, Dr Bassuk and her team started to identify and propagate those hybrid oaks that are better adapted to drought and climate change. To ascertain whether a plant is drought tolerant, scientists typically measure its ‘water potential at turgor loss’, In botany, turgor refers to the rigidity of plant cells due to the absorption of fluids. Plants with a negative turgor loss point are generally more drought tolerant, since they can continue to function over a wide range of water availability.

As part of this study, the team developed new methodologies to test how resilient her oak hybrids are in the face of drought. They did so at a molecular level by using a tool called an ‘osmometer’. Using these sophisticated instruments, the team are able to measure the osmotic pressure of the trees’ leaves (the pressure of water moving across a membrane due to osmosis). They can then see whether molecules found within the leaves cause the water pressure to increase. This process allows them to measure the ability of their tree species to survive in the dry, urban environment.

During this project, UHI has also been developing new ways to asexually propagate – or clone – their oak hybrids. They had successfully grown them from stool beds, a method that entails harvesting and growing shoots of suckers off of root stocks. However, this process was very slow, so they turned to tissue culture, or ‘micropropagation’, which basically involves growing tree cells in a tube.

Since 2017, tissue culture methods have allowed the team to speed up the process and generate large numbers of oak clones in one growing season alone. Their overall aim is to propagate their hybrids on a much bigger scale so that nursery growers can begin to grow and sell their own stock.

Trees growing in a plaza with CU-Structural Soil under the pavement in New York City

Mending Soil Woes

But what good is it to produce a better urban tree when soil conditions are so poor? No tree will thrive without adequate room to grow, nor will they survive for long in compacted soils. An example often cited by horticulturists is the 'bath tub effect', whereby trees are planted in compacted soils that can't drain water and their roots, unseen to the human eye, drown or rot. 'Compaction is probably the single most limiting soil condition for urban plants,' explains Dr Bassuk.

To prevent the effects of soil compaction, Dr Bassuk and her colleagues have developed a patented system known as CU-Structural Soil® or CU-Soil®, a precise mixture of soil and gravel that is specifically designed to go under sidewalks and pavement. The engineered soil allows for the compaction required to bear heavy loads, but at the same time has large pores that allow for tree roots to grow through it.

They have also developed another technique known by the playful name of 'Scoop and Dump'. In this system, they use a backhoe to dig in 'veins' of rich compost into poor, compacted soils. 'The backhoe scoops compost and compacted soil down to a depth of 18 inches and dumps it back on the ground, thus fracturing the compacted soil and allowing compost to settle between the clods,' explains Dr Bassuk. 'Plants are planted and mulched. The mulch is reapplied annually until there is canopy closure to replenish the organic matter in the soil. This technique has affected long-term soil remediation and improved plant growth.'

Along with soil compaction, the research team also need to consider other parameters of soil health. They have to take into account chemical parameters such as pH, phosphorous, potassium and salinity, along with physical characteristics, such as density, texture and the ability to hold and drain water.

Avoiding Transplant Shock

Once ideal trees have been identified and soils prepared, a final key issue to success is transplanting. Logically, Dr Bassuk is also addressing the best way to move their hardy new hybrids into the city to avoid transplant shock, which occurs when water supply is disrupted. This phenomenon, explains Dr Bassuk, results in the 'poor growth that trees often experience after transplanting including reduced stem growth and small leaves.'

Certain trees are more susceptible to this than others and in the past, nursery workers would often prune their roots before actually transplanting them. The thought then was that cutting roots would promote their regeneration and branching, while creating a denser root ball. But this practice involved hours of labour and fell out of favour with the invention of the modern tree spade. This powerful machine uses blades to cut a circle around a tree and then lift it and its encasing root ball and soil out of the ground.

However, tree spades haven't necessarily improved transplanting success for difficult trees like oaks, according to Dr Bassuk. Therefore, she and one of her graduate students are now investigating the age-old method of root pruning as a possibly better method.

They plan to prune the roots of several tree species and leave them to grow for one year in a nursery. Another control group with similar species will not be pruned. The researchers will then transplant both groups at the same time and start to record what happens. They will measure the hydraulic conductance in newly emerging roots, both at the time of transplanting and every month thereafter. Previous studies have shown that hydraulic conductance is linked to how well a tree responds to transplant shock. They will also measure growth of shoots, stems and leaves throughout the growing season. Dr Bassuk says that experiments are also under way to manipulate roots so that they can take up water more quickly, and increase transplant success.

Bassuk teaching students about woody pant Identification

Meet the researcher

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Dr Nina Bassuk obtained her PhD in Horticulture from the University of London in 1980. She has been a professor and program leader of the Urban Horticulture Institute at Cornell University for the past 37 years. She is a founding member and past president of the New York State Urban Forestry Council and is co-author of *Trees in the Urban Landscape*, a guidebook on establishing trees in disturbed and urban landscapes. Dr Bassuk has authored over 100 papers on the challenges of growing plants in urban environments, including how to select plants for difficult sites and improving soils and transplanting techniques. She works closely with municipalities to help implement best practices in urban forestry management and developed the Student Weekend Arborist Team to inventory public trees in communities in NY State. Dr Bassuk also teaches a course named 'Creating the Urban Eden', which integrates woody plant identification with landscape establishment techniques for difficult urban sites. She is a frequent invited speaker at national conferences and workshops.

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A GREENER FUTURE: LEVERAGING ECOSYSTEM SERVICES IN SUSTAINABLE LANDSCAPE AND CITY MANAGEMENT

As global climate change and other major environmental threats advance, scientists are looking for ways to evaluate sustainable solutions for energy, agriculture and city management. Ecosystem services are benefits provided to humans by nature, and over the past two decades researchers have begun refining ways to assess the value of these services compared to human-made options. **Dr Benedetto Rugani** and his team are developing novel ways to assess ecosystem services and advance the use of nature-based solutions in urban areas.

In 2005, after four years of rigorous research, the United Nations published the Millennium Ecosystem Assessment – an extensive review of the state of our planet's ecosystems. A popular term that emerged from the assessment is 'ecosystem services' – benefits that humanity gleans from nature around the world.

Ecosystem services are natural processes that support human economy and quality of life, such as harvesting fish from the sea or enjoying a hike in a national park. Generally, ecosystem services are divided into four categories: support services, such as nutrient recycling in the soil and production of carbohydrates by plants; provisioning services, which allow us to obtain food, clean water, raw materials, and energy from our environment; regulating services, which purify our air and water, sequester carbon, and break down waste; and cultural services, the functioning of ecosystems that we enjoy for recreation, science, and education.

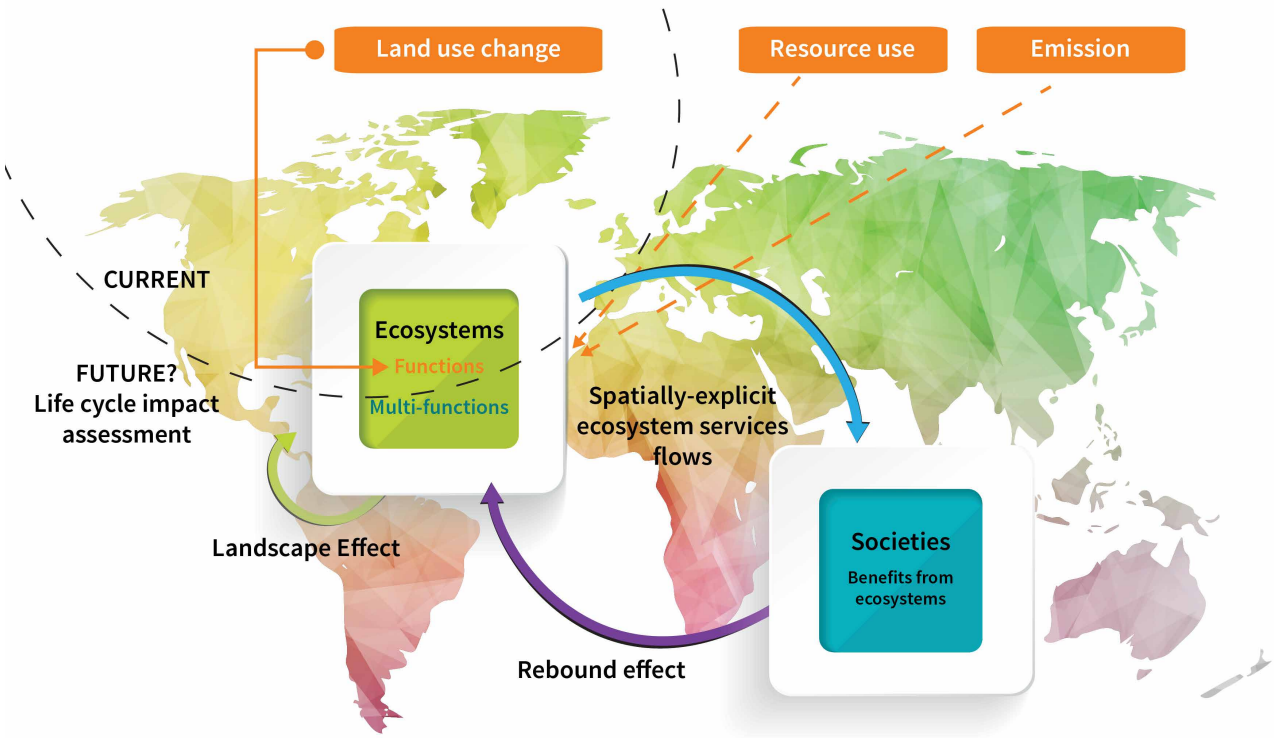
Valuation of ecosystem services, notably their monetisation, is becoming increasingly popular, as climate change and ecosystem loss threaten habitats worldwide. For example, some estimates put the agricultural value of honeybees and other insects pollinating crops in the US at $29 billion a year, underscoring the critical importance of protecting hive health. In a seminal paper from 1997, ecological economists estimated that the environment provides on average $33 trillion USD in ecosystem services worldwide every year (over $50 trillion in today's money), many of which would be nearly impossible for humans to replicate or replace even with modern technology.

One of the most recently proposed approaches to estimate changes in ecosystem services provision is by using the Life Cycle Impact Assessment (LCIA) – a cause-effect modelling framework that allows us to evaluate the impacts of human behaviour on a given ecosystem service. The problem with most LCIAs is that they focus narrowly and statically on single interaction pathways, while in reality, ecosystems are affected by a variety of dynamic relations and interacting factors. Habitats fluctuate in a multitude of ways that can mediate or amplify the effects of human activity, thus most LCIA models are limited in their ability to predict how a given change will impact an ecosystem service.

At the Luxembourg Institute of Science and Technology, Dr Benedetto Rugani and his close-knit team of graduate students, Benoit Othoniel, Thomas Elliot and Javier Babi Almenar, are engaged in developing multi-faceted measures of ecosystem services, improving LCIA methods and transforming land use planning, with a special focus on urban environments. Through their important work, the team hopes to contribute to a more sustainable future for humankind.

Placing Ecosystems in Context

The foundation of the team's work began in 2014, when the Luxembourg National Research Fund provided a grant for Dr Rugani to develop more rigorous LCIA frameworks. The VALUing Ecosystem Services for environmental assessment (VALUES) project employs novel methodologies to calculate the physical or monetary value of ecosystem services, in order to identify ways in which land managers and urban planners can most effectively utilise ecosystem services, while simultaneously protecting and preserving the ecosystem itself. The endpoint of VALUES is to create new scientific knowledge to advance the current LCIA practice for ecosystem services assessment.



Inspired by Othoniel et al., https://pubs.acs.org/doi/abs/10.1021/acs.est.5b03706

To meet these aims, the team predicted that they could take a page from the Multi-Scale Integrated Model for Ecosystem Services (MIMES), a framework that integrates the effects of multiple ecological and human activities on an outcome. This builds a more realistic picture of how complex environments operate and provides more robust predictions about how a particular activity is likely to impact an ecosystem in context.

Further, the team wanted to incorporate time-series data from both geographic information systems and statistical surveys to increase the resolution and representativeness of the model outputs. Accordingly, researchers can track changes in biodiversity, population dynamics, use of goods and services and land cover changes over time and create maps of ecosystem services over a region to identify areas that should be protected.

Utilising MIMES principles, the VALUES framework incorporates this data with features and knowledge from other modelling tools, to predict the impact of land use changes on pollinator activity and carbon sequestration in Luxembourg, and extending this approach to other ecosystem services in the European and global economies.

The integrated models developed through VALUES also allow researchers to predict changes in ecosystem service supply across different regions according to a multi-scale approach, thus supporting the development of more sustainable land management policies. For example, using the VALUES version of MIMES, one can assess whether land use changes in one country, including the demand for new productive land areas, can affect the delivery of ecosystem services locally and in other countries.

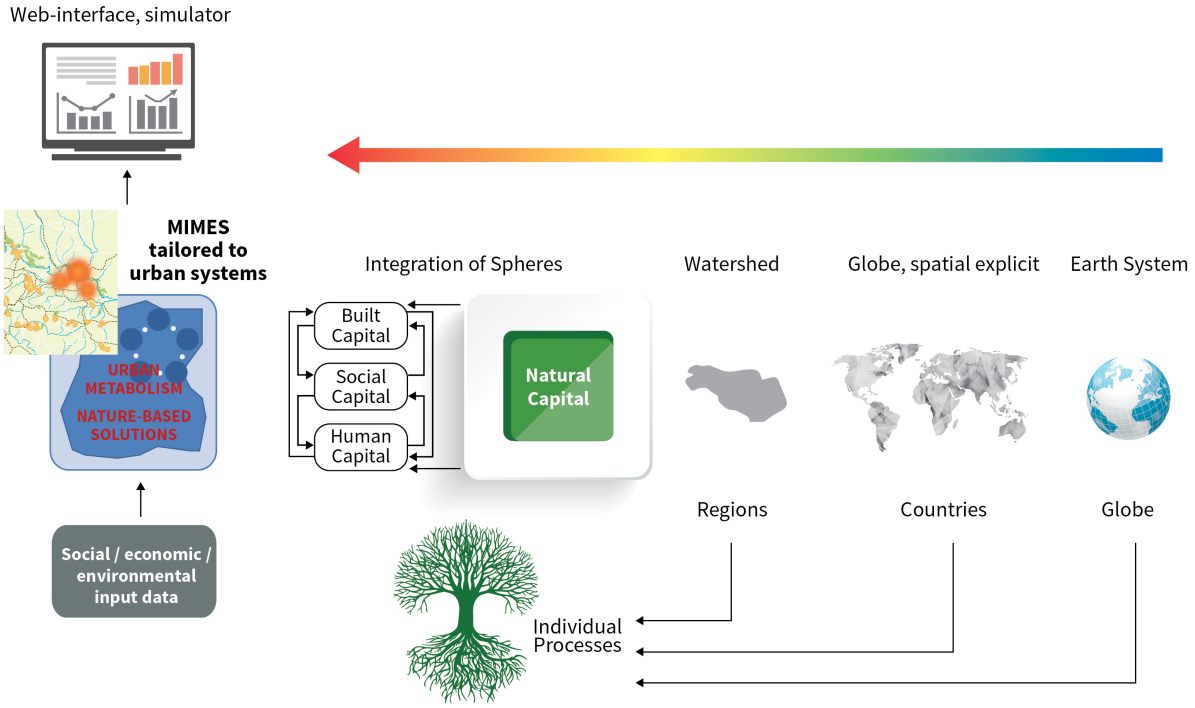
Estimating Ecosystem Impacts in Urban Areas

Because the city is where most of the population aggregates and consumes, and is where most environmental impacts are generated or solicited, the next logical step for Dr Rugani's team's work is to translate the findings of the VALUES project into practical tools that can aid urban planners and other built environment professionals in developing more sustainable cities.

Over half of the world's population live in major metropolitan areas, where large numbers of people and motor vehicles coupled with limited green areas often make for high levels of resource consumption and pollution, with potentially drastic effects on the health of city inhabitants and world climate as a whole. This is particularly true in developing countries, where less environmental regulation and older vehicles often converge to reduce air quality or increase noise pollution. A tool that could help urban planners and policy makers confidently plan high-impact nature-based solutions in urban areas could have a transformative effect on both the health of city residents and the global climate.

To understand city health on a grand scale, Dr Rugani and his team focus on what the scientific community calls 'urban metabolism', which describes the flow of materials and energy in a city, most often carbon. If city planners are able to accurately predict the effects of various ecological projects on the environmental and socioeconomic improvement of urban metabolism, they can make more informed decisions towards building healthier, more sustainable cities.

To this end, the team is developing ESTIMUM – the Ecosystem Services Toolbox – developed from multi-scale Integrated Modelling of Urban Metabolism. Building on the successes of VALUES, ESTIMUM utilises a system dynamics approach to simulate complex city environments under various conditions and situations, to determine which sustainability solutions will have the most positive impact across the widest breadth of climatic, environmental, and



Inspired by Boumans et al., https://www.sciencedirect.com/science/article/pii/S2212041615000054

technological scenarios. The tool will help city stakeholders simulate, monitor, and manage environmentally friendly ventures.

The team is currently testing a beta version of ESTIMUM with city planners in Esch-sur-Alzette in Luxembourg, Siena in Italy, and Lisbon in Portugal, to evaluate the performance of the tool across multiple climates and city sizes. So far, their results are promising.

For instance, the test model for Lisbon considers the synergistic effects of different transport modes, fuel types, population dynamics, and carbon and water cycles. This model simulates, under different scenarios, how in Lisbon urban trees provide both local and non-local ecosystem service co-benefits relating to carbon sequestration, heat island effect and water regulation. These preliminary findings allow for the quantification of net energy savings as a result of increasing urban trees, for example.

Bringing Nature to the City

Dr Rugani and his team recognise the powerful impact that natural solutions can have on cities and communities, not only in practical terms of ecosystem services, but also the psychological benefits that nature provides to city residents. One of their major goals is to help 're-nature' urban areas, but in order for cities to implement large-scale nature projects, there is a need for stakeholders to understand the potential costs and benefits of such a project, as well as a need for access to information and resources to facilitate sustainable urban planning.

The team's newest venture is framed within the European Union's Horizon 2020 programme, and is dedicated to evaluating and advancing nature-based solutions for city management in Europe, while creating a database of solutions that can benefit cities globally. To accomplish this, the team is partnering with numerous universities, research organisations, industry leaders, and policy makers to pilot the 'Nature4Cities' project in four cities across the EU: Milan in Italy, Çankaya in Turkey, Szeged in Hungary, and Alcala de Henares in Spain.

Over the next three years, Nature4Cities has four primary goals. First, the team will collaborate with other 25 partners to build a nature-based solution knowledge base that will be publicly available, and will include frameworks for analysing potential solutions. Second, they will use their integrated assessment expertise to develop a holistic assessment method that incorporates inputs from policy, business, and finance along with ecosystem services measures, to help identify the best solutions for each city's unique situation.

Third, they will adapt existing urban management processes to suit nature-based solutions, working to engage citizens and collect urban data that will drive re-naturing projects forward. Finally, the team will work with the four pilot cities to implement actual nature-based projects and assess how well their predicted scenarios align with real-life results.

Each of these goals will continue to build upon and inform the others. The knowledge base and analyses will continue to grow and be refined by contributions from partners and findings from the pilot cities. As governance models develop through the collaborative efforts of citizens, politicians, researchers, and industry stakeholders, best practices for facilitating participation will be added to the available resources.

A Sustainable Future

By understanding the dynamics and potential of ecosystem services, we can identify sustainable solutions for modern environmental problems. By developing resources to help cities understand how they can use nature-based solutions to enjoy healthier cities at lower costs, Dr Rugani's team is helping to fight climate change and build a greener future.

ESTIMUM and Nature4Cities are poised to become international resources that help cities leverage ecosystem services in novel ways that improve quality of life for residents and reduce pollution, creating a healthier, more sustainable world for everyone.




Meet the researchers

Benedetto Rugani

Dr Benedetto Rugani is a Research & Technology Associate at the Luxembourg Institute of Science and Technology, affiliated to the department of Environmental Research and Innovation, within the RDI Unit on Life Cycle Sustainability and Risk Assessment. During the last 7 years, Dr Rugani has contributed to the improvement and combination of existing methodological approaches and indicators to assess the environmental impact of life cycle activities on the supply of natural resources and ecosystems productivity. His current research and project management activity focuses on developing modelling approaches and decision support tools for assessing ecosystem services, with a focus on urban systems.

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Benoit Othoniel

Benoit Othoniel is an agronomic engineer and PhD researcher, particularly interested in the management of resources and land. After diverse experiences abroad, from working on palm populations in Madagascar, to irrigation systems in Cambodia, he started a PhD thesis on the assessment of land use and ecosystem services in life cycle assessment. By combining theoretical knowledge on ecology, economics and sociology in models, his aim is to develop tools and indicators that can support the design of sustainable development strategies at multiple scales, from local to global.

Thomas Elliot

Thomas Elliot is a PhD researcher at MIT Portugal in Lisbon, while also working with Dr Rugani at the Luxembourg Institute of Science and Technology. Originally from New Zealand, his professional background is in mathematical modelling and energy systems. His research interests include ecological economics, Life Cycle Assessment, and environmental ethics. His PhD topic incorporates ecosystem dynamics with urban metabolism in pursuit of enhancing decision support for sustainable urban planning. His work is inspired by an intrepid journey from New Zealand to Europe by bike and sailing yacht in the year following his Master's degree, during which he was exposed to some challenging negative social and ecological environments. Those experiences have shaped his philosophy towards strong sustainability.

Javier Babí Almenar

Javier Babí Almenar is a PhD researcher with BA and MA degrees in Architecture from the Polytechnic University of Valencia, a BSc in Environmental Sciences from the University of Valencia, and an MSc in Integrated Landscape Ecology from Cranfield University. He is interested in the study of socio-ecological systems, especially urban contexts, under a systems dynamics approach integrating knowledge and tools from urban metabolism, urban ecology, and life-cycle thinking. The chief purpose of his research is to better understand how to redesign urban systems in a more sustainable manner using nature-based solutions.

FURTHER READING

VALUES – VALUing Ecosystem Services for environmental assessment. More information available at: http://www.lifecycle-values.lu/; National Research Fund Luxembourg (FNR; C13/SR/5903117).

ESTIMUM – Ecosystem Service Toolbox developed from multi-scale Integrated Modelling of Urban Metabolism. More information available at: http://www.list.lu/en/project/estimum/; National Research Fund Luxembourg (FNR; C16/SR/11311935).

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BUGS ARE FRIENDS: TAKING AN ECOSYSTEM VIEW ON FOREST HEALTH

Humans commonly view insects as unwanted nuisances, and many modern land-management practices focus on reducing insect numbers using toxic pesticides and invasive biological controls. **Professor Timothy Schowalter** at Louisiana State University has been studying insect communities for decades, and suggests that by working with insects in an ecosystem approach, we could enjoy healthier environments for years to come.

They Outnumber Us

Arthropods, the animal phylum including six-legged insects and their many-legged relatives such as spiders, are the most abundant type of animal life on Earth. Over one million arthropod species have been identified, but scientists estimate that there are between 10 and 30 million species currently living on Earth.

Arthropods are almost always viewed as pests, even though the majority are harmless to humans and much of our food supply depends on pollinators such as honeybees. Professor Timothy Schowalter at Louisiana State University has been studying arthropods over his lengthy career, and his research suggests that even species commonly viewed as pests can be healthy for ecosystems under the right conditions, and outbreaks should be viewed as symptomatic of larger management problems. He argues that when humans work with insect communities, rather than against them, we have much to gain.

Professor Schowalter's work is driven by two main questions – how do arthropod communities respond to environmental changes, and how do changes in these communities alter ecosystem conditions and the ability of an ecosystem to deliver ecological services? To pursue answers to these questions, Professor Schowalter and his colleagues have engaged in large-scale arthropod diversity research projects in forests all over the world – from the chilly boreal conifer forests of northern latitudes, to the species-rich tropical forests of Puerto Rico. Their work has revealed new insights into how arthropods both respond to and drive changes within forests, and how humans can work with these abundant creatures to support healthy forests and ecosystems.

The Living Forest

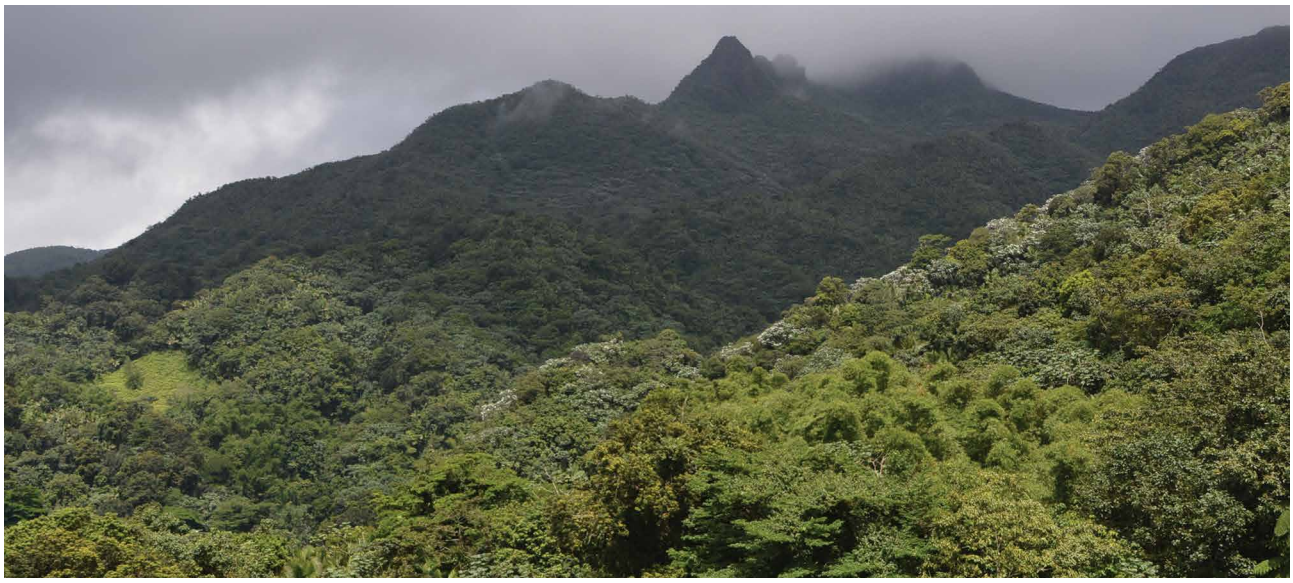
Understanding how arthropod communities respond to changes in a forest can help land managers understand how to keep forests healthy and thriving. Because of their small body size and environmental sensitivity, arthropods often act as early indicators of an ecosystem problem. Arthropod diversity is often used as a measure of the health of an ecosystem – particularly in the soil, where insect abundance and diversity are often more reliable measures of soil health than standard chemical assays.

Professor Schowalter and his team focus on insects living in the canopies of trees, and they have assessed arthropod diversity in many forests worldwide. One area of particular interest to the team is arthropod responses to tree harvesting methods in timber forests. The historical practice of 'clear-cut harvesting' – taking all the trees in an area at once – has had dramatic effects on forest ecology in the Pacific Northwest region of the United States and elsewhere, leading to declines in forest biodiversity and an increase in soil erosion. As an alternative, modern harvesting practices involve removing trees in gaps, clearing around clusters of older trees, and removing trees sporadically throughout a forest, while leaving some older trees evenly dispersed.

CREDIT: Professor Chow-Yang Lee (University of Malaysia)

Professor Schowalter's team compared the effects of these methods on canopy arthropod diversity in forests in Oregon and Washington, taking samples of arthropods a year before harvesting and once a year for two years after. They found that differences were minimal between treatments, but other studies in older forests treated in similar ways indicate that these differences grow more dramatic over time. The team's results suggest that arthropods may be more sensitive to large scale changes than local events, though these effects must be tracked over time.

‘If insect pollinators and decomposers disappeared, humans would quickly notice that most fruits and vegetables would no longer be available, and plant and animal wastes would accumulate and probably bury us.’



Professor Schowalter is also engaged in understanding the effects of the environment on arthropods in tropical forests. His team has been involved with the Luquillo Experimental Forest Long-Term Ecological Research Project in Puerto Rico for over 20 years, where they have been experimentally manipulating sections of forest, and observing arthropods’ natural responses to weather events such as hurricanes.

Using the resources of the Long-Term Ecological Research Project at Luquillo, the team’s experiments showed that the composition of arthropod communities fluctuates following a storm, and so they went on to investigate what storm factors contribute most to these fluctuations. The two major factors they were interested in were canopy openings and debris deposits. Canopy openings occur when many tall trees or branches fell in an area, allowing more sunlight and rain to reach the forest floor, while debris deposits are the leaves, twigs, and branches that fall off plants during a storm and end up on the ground. To replicate these conditions, the researchers created four forest plots – one untouched, one where they trimmed branches to create a canopy opening, one where they deposited debris, and one where they did both. While none of the arthropod groups in the forest responded to the canopy opening alone, nearly half of these groups showed changes in abundance in response to debris or the combined condition. This suggests that immediate biotic (living) factors, such as debris, lead to a more direct change than abiotic (non-living) factors, such as increased sunlight.

During Professor Schowalter’s time in Puerto Rico, his team has also observed insect responses to natural events – particularly the highly destructive hurricane Hugo in 1989 and Georges in 1998. Similar to their experimental results, the team found that about half of the studied arthropod groups responded strongly to storms, usually related to their niche in the forest. For example, insects that feed on detritus were most abundant immediately after a storm, while those that feed on tree sap surged in gaps when new growth emerged. They also found that when a group was severely reduced following a storm, they were unlikely to fully recover for many decades. ‘Some insect species can become very abundant, whereas others may decline or disappear, as a result of any particular change in environmental conditions,’ Professor Schowalter explains. Understanding how storms can impact biodiversity in forests grows more important as climate change increases the number and intensity of storms every year.

Unlikely Allies in Forest Management

Despite their bad reputation, many insects provide critical ecological services to their habitats that benefit humans, such as decomposition, pollination, prey for other animals, seed dispersal, and even herbivory. As decomposers, insects help to accelerate the breakdown of dead plants, plant parts and animals, helping to return organic nutrients to the soil. For example, burrowing insects like ants often carry organic matter deep into their nests and contribute to soil turnover, both of which lead to healthier soil that is better suited to grow plants. Pollinators are a critical component of plant life on earth – many plants cannot reproduce without pollinators and some plant–pollinator relationships are highly specific. For example, more than a third of our crop varieties depend on honey bees and other insects for pollination before a fruit or vegetable can be produced.

Insects also form an important food source for many other larger insects and animals, including humans. For example, salmon populations are supported by freshwater arthropods during their early years – thus, a healthy salmon population depends on a thriving arthropod population. Insects also operate as dispersal agents for other organisms, transporting seeds, fungal spores, and sometimes diseases. Ants are particularly important in this role, as they often carry seeds deep into the ground where they can begin to grow under ideal conditions. Many human societies acquire substantial portions of their protein from insects.

Insects also act as herbivores by eating portions of plants – an underappreciated ecological service. While excessive munching can be bad for a plant, research has found that many plants easily recover from a little nibbling, and in many cases, they are actually more productive after experiencing some herbivory. Some plants respond to an insect eating their leaves by investing more energy in their flowers and fruits, and this often benefits farmers' yields more than the application of pesticides. In the majority of cases, working with arthropod communities actually benefits humans. As Professor Schowalter explains: 'If insect pollinators and decomposers disappeared, humans would quickly notice that most fruits and vegetables would no longer be available, and plant and animal wastes would accumulate and probably bury us.'

He suggests that insects most often become problematic when the natural order of the forest is disrupted by human management practices. For example, outbreaks of the southern pine beetle can be devastating to pine groves, but primarily when when these fire-adapted habitats have been fire suppressed or planted too densely by humans. Under natural conditions, the beetles only attack weak and ailing trees or those growing close together, decreasing resource competition for healthy trees and creating niches for other wildlife. However, in fire-suppressed forests and plantations, trees grow much closer together and the short-ranged beetle can multiply far beyond its normal capacity. Beetle outbreaks are more a symptom of poor management practices than a feature of the beetle itself. Similar issues are seen in monoculture farming practices – due to a lack of plant and arthropod diversity, insects that would normally be kept in check by predators, or the limited availability of suitable hosts, are able to easily jump from plant to plant, quickly consuming an entire field. 'More diverse crop mixes and natural corridors for predaceous arthropods and birds have demonstrated the ability to reduce crop pests, whereas crop monocultures and agricultural intensification have the opposite effect,' Professor Schowalter says.

The team's work in this area suggests that even herbivorous insects play a major role in maintaining healthy forests. In Puerto Rico, they sought to understand how common herbivore effects help to fertilise the soil. Herbivores alter nutrients in soil by dropping 'greenfall' (foliage fragments that fall as an insect chews through a leaf), creating faecal waste known as 'frassfall', and increasing rainwater's ability to leech nutrients from leaves. The researchers simulated each of these conditions by enriching sections of the forest floor with only one of these substances and measuring changes in key soil nutrients over time. They found that frassfall created the most dramatic nutrient upticks and impacted other forest floor processes such as decay rates.

Perhaps unsurprisingly, the team demonstrated that herbivorous insects are a critical component of maintaining nutrient availability in tropical forest soil.

In the temperate forests of North America, insects are equally important. Professor Schowalter has taken an interest in rare old-growth forests that often act as habitats for species not found in younger forests. These old forests maintain more stable temperatures and humidity, and feature more diverse plant life and habitat niches. They also seldom experience the insect outbreaks and other problems associated with younger groves, and are in many ways protected by their incredible biodiversity.

Old-growth forests contain more diverse arthropod communities that are just as critical for nutrient cycling through herbivory and decomposition as they are for pollination and prey. They represent the pinnacle of a healthy forest that self regulates, and are critical to protect, both for the preservation of biodiversity and so we can learn valuable lessons from observing them. Forest management officials save time and resources when they learn to work with insects to manage groves, rather than embark upon efforts to suppress them as unwanted pests.

However, Professor Schowalter points out that in some cases, insects will need to be managed or controlled in the short term. 'For example, the droughts in western North America have stressed forests and led to catastrophic mortality due to fires and bark beetles, as a result of high vegetation density and fuel loads resulting from a century of fire suppression,' he explains. 'The warming and drying conditions of the region exacerbate the likelihood that if we let fire and insect outbreaks run their course, virtually all older forests could be burned. These forests, and others, need to be restored to conditions that were less prone to catastrophic wildfires and insect outbreaks. However, until these efforts make forests less vulnerable to fire and bark beetles, fire and insects will need to be managed to avoid catastrophic fires and outbreaks.' By adapting our management practices, we can stop inducing these insect outbreaks and fires in the future.

Lessons to Learn

Professor Schowalter's work highlights the diverse and complex roles that arthropods play in building and maintaining healthy ecosystems. Far from pests, these tiny animals are critical members of forest communities that can teach us valuable lessons about taking care of the land if we pay attention.

Meet the researcher

Professor Timothy D. Schowalter
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Professor Timothy Schowalter received his PhD in Entomology from the University of Georgia in 1979, following a BA in Biology and Anthropology from Wichita State University and an MS in Biology from New Mexico State University. He completed a post-doctoral position at Texas A&M University before joining the Department of Entomology at Oregon State University as an Assistant Professor in 1981, where he was eventually promoted to Professor and Interim Head. In 2003, he joined his current position with the faculty at Louisiana State University as a Professor of Entomology where he also served as the Department Head until 2015. Professor Schowalter is the author of four books, more than 70 peer-reviewed articles, and many book chapters and other publications. He served as Program Director for Ecosystem Studies at the National Science Foundation 1992–1993, as Vice President for Public Affairs for the Ecological Society of America (1999–2002) and as a Governing Board member for the Entomological Society of America (2013–present). He currently serves on the Editorial Boards for Frontiers in Ecology and the Environment and Journal of Economic Entomology, and was named a Fellow of the Ecological Society of America in 2012. His research focuses on insect responses to natural and anthropogenic disturbances, and the effects of these responses on ecosystem structure and function.

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SUSTAINABLE AGRICULTURE

SUSTAINABLE AGRICULTURE FOR OUR CHANGING WORLD

Our previous section showcased the efforts of many dedicated conservation scientists, each striving to protect and restore natural ecosystems that have been damaged by human activity. One of the most significant ways that humans have negatively impacted the planet is through our agricultural systems. In fact, agricultural land comprises almost 40% of Earth's total land area, with about one quarter of this being cropland and the remainder taken up by livestock and the crops needed to feed them.

Using up such a massive chunk of the Earth's surface to feed our ever-growing human population has had catastrophic consequences for the millions of species we share the planet with. In addition to the habitats that are destroyed when wilderness areas are converted to farmland, the use of fertiliser, pesticides and herbicides, which are toxic to many species, has further exacerbated the negative influence of agriculture on biodiversity.

To make matters worse, clearing CO_2-capturing forests to make way for agricultural land, and the greenhouse emissions associated with agriculture (primarily livestock) are both key drivers of climate change. This, in turn, further reduces biodiversity through rising temperatures and extreme weather.

With the human population expected to reach eight billion by 2024, and nine billion by 2042, even more food will need to be produced. The environmental impact of this could be catastrophic, with more wilderness and forested areas being converted to agricultural land. Reducing our reliance on meat and dairy will certainly help matters, as even though we dedicate about three times more land to rearing livestock than growing crops, meat and other animal products make up less than a fifth of our caloric supply. Therefore, even a modest shift towards eating less meat will have a great impact.

Cultivating crops in a more sustainable fashion, such as reducing our reliance on pesticides and herbicides, will allow biodiversity to thrive in agricultural settings. Also, developing crop varieties that are more resilient in the face of climate change, which threatens our food supply through droughts, flooding, and other extreme weather events, will also help to boost yields, reducing the need for land.

Therefore, many scientists are exploring these avenues, to work towards a future in which our agricultural practices are more in tune with nature, while also ensuring food security for our ever-increasing human population. In this section of the edition, we showcase several promising developments towards this aim.

In our first article of this section, we meet a multidisciplinary team of scientists from Michigan State University, South Dakota State University, Iowa State University and North Dakota State University, who are interested in fostering sustainable agricultural practices in the Great Plains of the US. Poor decision-making regarding land usage in these regions was behind the infamous dust storms of the 1930s, which greatly damaged the ecology and agriculture in this region. Headed by Dr David Hennessy, this research team is currently working to provide a scientific foundation upon which decisions about land use can be made. In particular, they are interested in grassland conservation, which provides invaluable habitat for a multitude of species.

Continuing on the topic of grasses, and the ecosystems they provide, our next article features a simple, yet highly effective way to grow crops in a sustainable, environmentally-friendly fashion. Here we meet Dr Sangamesh Angadi and his colleagues at New Mexico State University, whose new system involves interspersing crops in circular pivot fields with rings of native grasses. The presence of these native grasses is likely to support insect predators, which will reduce pest numbers, thus reducing the need for pesticides. In turn, the reduced need for pesticides will boost pollinator numbers and increase the local biodiversity. These grasses are also excellent at sequestering carbon dioxide from the atmosphere.

Finally, grasses also help to trap rainwater that would normally be lost to evaporation, reducing the amount of irrigation that the crops need by around 20%. Since crop irrigation uses up the majority of the

freshwater that we withdraw from groundwater, rivers and lakes, this reduced need for water will make a huge impact. Also, with freshwater supplies in swift decline due to the effects of climate change, strategies to reduce our consumption are becoming increasingly vital.

Another researcher who is exploring ways to reduce the amount of water needed for crop irrigation is Dr Dedrick Davis at Alabama A&M University, who we'll meet in the next article of this section. In one of his ongoing projects, Dr Davis is investigating how adding biochar (a by-product of making biofuel) to soil might improve its ability to retain water, reducing the amount of irrigation needed.

Also passionate about water conservation is Dr Maureen McCarthy at the University of Nevada and the Desert Research Institute. Her research team works with Native American communities in arid regions of the US, whose water supply is particularly threatened by climate change. Their aim is to leverage both native wisdom and western science to enhance tribal agricultural resilience in the face of water shortages and extreme weather events caused by climate change.

Next, we meet a team of three crop scientists at Kansas State University, Dr Robert Aiken, Dr Vara Prasad and Dr Schlegel, who are also working to increase our agricultural resilience in the face of climate change. The team works with some of the world's most important food crops, including wheat, rice, corn and soybean, to improve their tolerance to drought and heat. The team's new cultivation strategies will be vital in the process of adapting agricultural systems to the impacts of climate change, particularly in semi-arid regions.

Adapting our staple crops to the deleterious consequences of climate change was the focus of a recent conference entitled 'Transitioning Cereal Systems to Adapt to Climate Change' (TCSACC). Bringing together experts from five continents, the aims of this conference were to strengthen the global network of researchers addressing the impacts of climate change on semi-arid cereal-based systems, to share approaches and to facilitate multidisciplinary collaboration towards accelerating climate change resilience in cereal systems, thus advancing global food security. In the next article of this section, we speak with one of the organisers of the event, Dr Sanford Eigenbrode, who discusses some of the key outcomes.

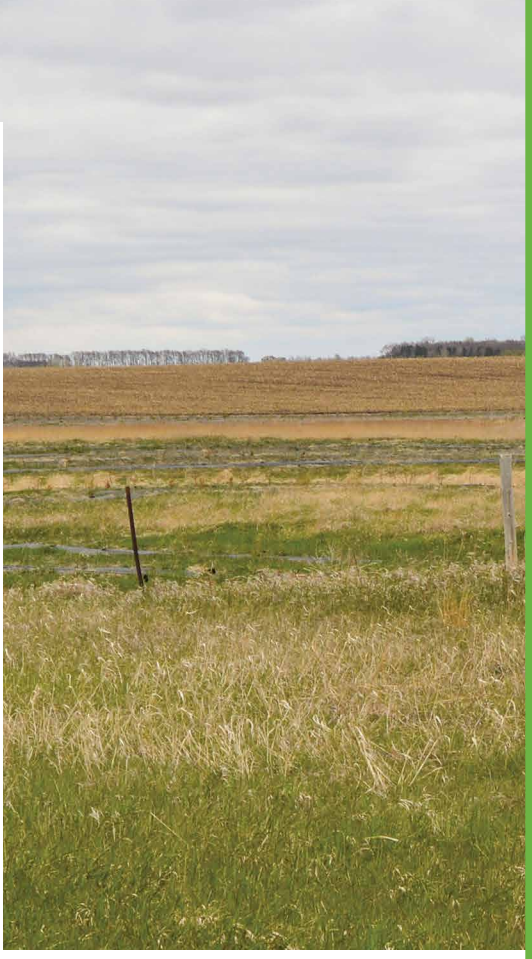
In the next article of this section, we highlight a nutritious crop that is surprisingly tolerant to extreme environmental conditions – the cowpea. Not only does this ancient crop show excellent climate resilience, but it has also been shown to support pollinators and improve soil quality, making it well placed to improve the sustainability of modern agricultural systems. Here, we meet Dr Louis Jackai, Dr Beatrice Dingha, Dr Mulumebet Worku and their students at North Carolina A&T State University, who are carrying out research that could open up wider avenues for the cultivation of this versatile crop.

Another sustainable crop that has been cultivated since antiquity is apio – a tuberous crop resembling a plump parsnip, which presents celery-like shoots that grow up to two metres in height. Unfortunately, over the past 15 years, apio farmers in Puerto Rico have experienced devastating revenue declines, perpetrated by a disease known as 'corm rot'. In the next article, we meet Dr Martha Giraldo and her research team at the University of Puerto Rico, who collaborated with local farmers to investigate the source of this disease. By investigating the range of different microbes present on the plant, the team identified that the root cause is a nematode species called *Rotylenchulus reniformis*. This finding allowed Dr Giraldo and her colleagues to develop crop management systems for effective disease control.

In our final article of this section, we introduce Dr Philippe Rolshausen and his team at the University of California, who also investigate the microbial communities that live on and in crops. In particular, the team studies fungal and bacterial diseases that affect the vascular systems of grapevine and citrus. Just like Dr Giraldo, Dr Rolshausen works to understand the root causes of these diseases, so that effective strategies be implemented.

THE FARMER AND THE RANCHER

A collaborative effort driven by researchers from Michigan State University, South Dakota State University, Iowa State University and North Dakota State University, aims to improve the age-old art of farming.



Vast, dry, and flat. The Great Plains of the continental US stretch from the foot of the Rockies through to the grasslands by the Mississippi, as far north as Canada and as far south as Texas. Covering 1.3 million square kilometres (a size roughly equivalent to Peru), it is renowned for being an immense expanse of farms and paddocks, with a tree, a creek or a town thrown in for variety every so often.

Hot summers and cold winters, gale-force winds and dust-storms. The hard climate can mask the vital importance of the Great Plains in the agricultural economy of the United States. From the earliest days of settlement, ranchers drove their herds across the plains towards food and water – a migration which opened up the land for later homesteaders and settlers. Today, almost 80% of the land is used for crops and pastures, while one in every five American cows can be found grazing in the Dakotas or Texas.

Despite this, many challenges will be missed by tourists as they travel from north to south through rolling fields of corn, wheat and cattle. The region is sparsely settled, with large portions having population densities of less than 2 people per square kilometre. Population decline is exacerbated by growing migration of many towards the wetter coasts, and many thousands of abandoned homesteads can be found scattered throughout the Great Plains where settlers simply packed up and left. The most notorious of these migrations occurred in the 1930s, when excessive ploughing of the fields led to runaway erosion and brought 'the Dust Bowl' into the public consciousness.

The Dust Bowl disasters were in part brought about by a failure in land use – the manner in which the area is managed for both human and environmental requirements. Although land use is a decision to be made by farmers and public authorities, there is rarely a simple answer as to how any one particular piece of property should be used. Instead, several competing priorities must be balanced, be they economic, social, political, or environmental.

A Farmer's Dilemma

An example of this multi-faceted decision-making is the choice between grassland and cropland. Grassland is, as the name suggests, land that is predominantly covered with various grass species and is largely used for grazing cattle. Cropland, by contrast, is land that has been prepared and then used to grow crops, such as corn or wheat. Grassland and cropland have different advantages and disadvantages, and farmers need to decide which approach is best for their particular circumstances.

In recent times, the appeal of cropland has significantly increased, supported by a variety of factors. Agricultural breeding and biotech developments are providing more drought-tolerant and pest-resistant crop varieties than in times past. Crop profits have been well above average for much of the last decade, partly due to demand from emerging markets but also partly due to government subsidies such as crop insurance and renewable energy mandates. Farmers respond to these incentives by increasing crop production – the amount of North Dakota covered by corn, for example, has risen ten-fold to over two million acres in the last 50 years.

These changes have implications beyond the farmers' markets. Grassland ecosystems support many diverse bird and animal species, and changes toward crop rotations that emphasise corn and soybeans reduce the available habitat. Excessive conversion of grasslands to croplands can negatively affect the entire region and thus needs to be approached with care – where care requires that the reasons for these choices be thoroughly understood.

‘Understanding land use in the Northern Great Plains is both important for public policy and a technically challenging endeavour’

Converting for a Reason

It is this knowledge gap that Dr David Hennessy and Dr Hongli Feng and their colleagues – including Dr Adnan Akyüz, Dr Larry Janssen, Dr Tong Wang, Dr Mike Wimberly and Dr Peter Wolter – wish to fill. An interdisciplinary team drawn from several institutions across the United States, they aim to provide the scientific foundation upon which further decisions about land use and conversion can be made.

We asked Dr Hennessy about the problems farmers faced when making these decisions. ‘The weather cycles and implications from climate change pose many challenges. Due to soil type and weather, much of the land has been either unsuitable for or marginally profitable in crop production,’ he commented. ‘Some programs operated by the federal government seek to promote grass-based land use, while other federal initiatives have made cropping more profitable. Technological advances have also had differential impacts on the yields and production costs of major crops.’

A survey-based approach taken by the team has been very successful in gathering information on agricultural choices. Responses from farmers showed that almost 40% had created some form of grass-to-cropland conversion on their land, with the area converted totalling over 5% of the land under ownership. The data showed that this conversion was most common amongst large farms, particularly when owned by younger farmers or those with higher agricultural income. Their conclusions, set out in a recent publication, noted that the current level of government conservation programs was not sufficient to maintain the existing grassland ecosystem.

This kind of insight would be impossible without the ability to gather opinions from farmers across the region. ‘Landowners in the area have been very open and generous with their time,’ says Dr Hennessy. ‘We have been most fortunate to have obtained great cooperation and insights about how long-term land decisions are made and why they are made.’

Survey information can also be merged with further data to provide detailed models of the Great Plains region. This is, unfortunately, a long and difficult process. ‘Available data on land use are problematic,’ admits Dr Hennessy. ‘Much of the work has been to better understand existing data through computer analyses. We have also spent time collecting new data and bringing together dispersed data.’

Difficult, yes, but certainly achievable. Previous work by the group has brought together data sources as disparate as temperature records, satellite images and county alfalfa harvests – using these in combination to help identify the manner in which farmers have shifted between different crop types in response to economic and climatic pressure. This work has led to publications and presentations, and has been particularly prized by farmers and others looking for support with their long-term planning decisions.

The Easement Problem

Land-use decisions are not just a matter of economics, but also one of government policies. Large tracts of grassland in the Northern Great Plains are prized by conservationists and duck hunters because these lands provide the optimal mix of grass cover for duck nesting and wetlands for duck feeding. Duck hunters and others pay annual 'duck stamp' fees to the US federal government, who use the money to buy grassland easements – a legal agreement entered into by the landowner and the government, in which the landowner is paid to permanently keep their field in a grassland state. In other words, the right to convert to cropland in the future is given away in exchange for a lump sum payment. This is an advantage for farmers who need to maintain a viable operation and would prefer to stay in grass-based agriculture (as it adds an extra bit of cash) but represents a difficult decision for others – is the money worth losing the chance to change at a later stage?

The difficulty of this discussion is not helped by steady improvements in crop yields and input-saving technologies, which make thoughts of cropland conversion yet more tempting. This in turn means that the government must offer greater sums of money before farmers will accept a grassland easement, which creates problems for already-stretched budgets. Yet governmental budgets are complex creations, with constraints both on the amount of money that can be spent and the timing – for example, can leftover cash to buy easements be saved for next year or must it all be spent this year? These constraints make the government's already involved decision on easement payments even more complex.

To help with sorting through alternative strategies for conserving grassland, the group has developed models of the decision-making process. By modelling the choices available to the farmers themselves, they can identify the ideal option for the farmer under several different circumstances. In combination with a model of the differing budget approaches, this can then be used by government agencies to identify their 'best value' investments. In other words, where the government can use their minimal budget to maximise the uptake of grassland easements.

Cows Eating Their Veggies

The quality of the grasslands is also dependent on the manner in which they are used for cattle grazing. Many cattle grazing operations practice continuous grazing, whereby livestock are left to graze over one large area for much of the season. The approach needs low investment in fencing and the provision of water. However, poorer pastures often result – the animals will eat more of the plants they like and leave the weeds to grow unchecked, the manure will be concentrated in patches rather than spread widely, and certain areas will bear the majority of hoof-based erosion.

To combat this, a more labour-intensive method known as Management Intensive Grazing (MIG) is used. The available land is divided up into a large number of paddocks, and livestock are moved between each paddock every couple of days, with a correspondingly long recovery time for all other paddocks. Cattle can be picky eaters, and if they pass over some grass today it will be less tempting tomorrow and succulent new leaves won't have the opportunity to emerge. By ensuring that cattle eat even the non-tasty plants, and then providing paddocks with a chance to recover from grazing, MIG is able to improve stock yields and reduce weed levels.

Despite this potential, the uptake of MIG in the United States has been limited. Why is this the case? Farmers make decisions on land use based on a number of different factors, with a major goal being to (sustainably) maximise the money they can make from their land. Although MIG appears to have benefits, there is a corresponding need for additional work and investment – both factors that may put farmers off.

To provide much-needed data on the effect of MIG, Drs Feng, Wang, Hennessy and collaborators have begun a new research project covering the impact and uptake of MIG, with five main goals. The first is to determine the short and long-term economic impact of MIG. The second goal involves assessing the environmental impacts of using the grazing method. The third is to see if MIG is associated with reduced grassland-to-cropland conversion rates. Identifying the factors affecting MIG uptake is the fourth goal, and finally, the team wish to take all of the information gathered and use it to improve current government programs that aim to promote MIG.

'Our analyses of data are still ongoing,' commented Dr Hennessy, 'but we are seeking to deepen and broaden what we have already learned. It is clear so far that motives for land use have emerged which have not received much attention among policy makers and researchers. For example, while financial incentives matter, farmers generally are very attached to their land and take stewardship very seriously.'

The ideal use of land in the Great Plains is dependent on a complex web of interlocking factors. Unravelling this web, through studies such as those done by Dr Hennessy and his colleagues, ensures that decisions are made on a solid scientific basis, considering every economic and ecological factor.

Meet the researchers

Dr David A Hennessy
Dr David Hennessy has long-standing interests in the fields of agriculture and economics. His particular focus is on the production economics of agriculture, together with its linkages to agricultural finance, technology adoption, food and food safety, and the environment. With a PhD from Iowa State University and a career spanning several institutions, he is currently a Professor and Elton R. Smith Chair in Food and Agricultural Policy at the Department of Agricultural, Food and Resource Economics, Michigan State University.
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A Professor of Economics at South Dakota State University, Dr Larry Janssen has spent his research career examining the effect of financial and policy decisions on farmland production and economics. Having supervised a number of students and published more papers than can easily be counted, he is a highly respected member in the field of agricultural economics.
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CIRCLES OF HOPE: CIRCULAR BUFFER STRIP SCHEMES FOR AGRICULTURE

Agriculture in the semi-arid US Southern Great Plains depends on irrigation water from the Ogallala Aquifer, the largest in the country. With the aquifer in decline, new ideas will be needed to ensure the future of irrigated agriculture in the region. **Dr Sangamesh Angadi** and his colleagues at New Mexico State University have come up with a simple, cost-effective system of interspersing crops in circular pivot fields with rings of native grasses, to buffer against the effects of damaging winds, improve water cycle, reduce soil erosion, and improve ecosystem services.

Improving agricultural productivity is essential to feed the world's growing population. One of the limiting factors to achieving this is the availability of fresh water, itself under increasing pressure from human demands. Climate change exacerbates the problem, leading to more frequent droughts and erratic rainfall patterns. Simultaneously, intensive agriculture leads to environmental degradation and the loss of valuable ecosystem services. In the semi-arid agricultural region of the United States Southern Great Plains, one agronomist believes his idea could be part of the solution to these complex challenges.

From Prairie to Dust, and Dust to Fields

The Southern Great Plains was once a prairie, grazed by roaming herds of bison. When settlers arrived, they ploughed the native grassland to grow crops. Intensive cultivation approaches, together with drought, led to the loss of the grass cover and damage to soil structure. In the absence of the moisture trapping properties of grass and deep root systems, the region's strong winds and sporadic, intense rains severely eroded the soil. The 1930s saw devastating dust storms in the region, known as the Dust Bowl. Salvation came in the form of new irrigation techniques, which allowed farmers to draw on the Ogallala Aquifer – an ancient underground lake – transforming the region into one of the most productive parts of the US, where agriculture is a vital part of the local economy.

The aquifer currently supports over 5.8 million hectares of agricultural land, covering the Midwestern landscape with green circles of crop growth produced by centre pivot systems, which irrigate the circular field. For the local population, it is also an important source of household water. However, the aquifer is only replenished slowly, by rainfall and snow melts that are becoming increasingly scarce due to climate change. It is now overexploited, and within a few decades, 35% of the irrigated land in the area could be dryland.

Soil erosion, typically held in check by irrigation, is now on the rise. Dust storms have returned to the region, and local farmers fear a repeat of the Dust Bowl could be on the way. Because of the water shortage, they are increasingly forced to restrict the area of land they can irrigate, resulting in 'partial pivots', with a sector of their circular crop fields remaining unirrigated. High rates of evaporation from the irrigated topsoil, especially early in the growing season, mean that more than 50% of the water applied is lost to the atmosphere. Crop plants at the edge of the pivot are particularly vulnerable to the harsh conditions, and are often shorter than the rest of the crop, or may even be killed.

Prairie Grass as Protective Circular Buffer Strips

Dr Sangamesh Angadi is an agronomist at New Mexico State University in Clovis, based in the Department of Plant and Environmental Sciences and Agricultural Science Center. He and his colleagues believe that the natural prairie ecosystems that dominated the Southern Great Plains in the past could hold the key to the future of agriculture in the region.

‘There is a need to assess linkages between the Food-Energy-Water components of CBS and develop quantitative relationships to advance this technology to large scale adoption in the Southern Great Plains’

The grasses protect the land from soil erosion in several ways: their deep root systems anchor the soil in place. They also have the ability to trap water from rain and snow, gradually transferring it to the soil, and buffering topsoil from the effects of heavy rain. By acting as wind barriers, they reduce the evaporation of soil water, and create more humid microclimates.

Strips of vegetation planted alongside crops are called buffer or wind barrier strips, with wind protection most pronounced when the strip is at a right angle to the direction of the wind. These strips show great benefits for agriculture in windy climates, reducing crop water consumption and improving yields, through effects such as reducing evaporation and damage to seedlings by windborne soil particles. Although research into this effect in irrigated crops has been limited to date, a New Zealand study found that wind breaks reduced water use by up to 20%.

Dr Angadi and his research team came up with the idea of reintroducing elements of the original prairie grassland into centre pivots, regaining their protective functions and improving productivity. This involves redesigning the partial pivot plot, turning the unirrigated portion into circular buffer strips of native perennial grasses and forbs – CBS for short – interspersed with circles of the crop.

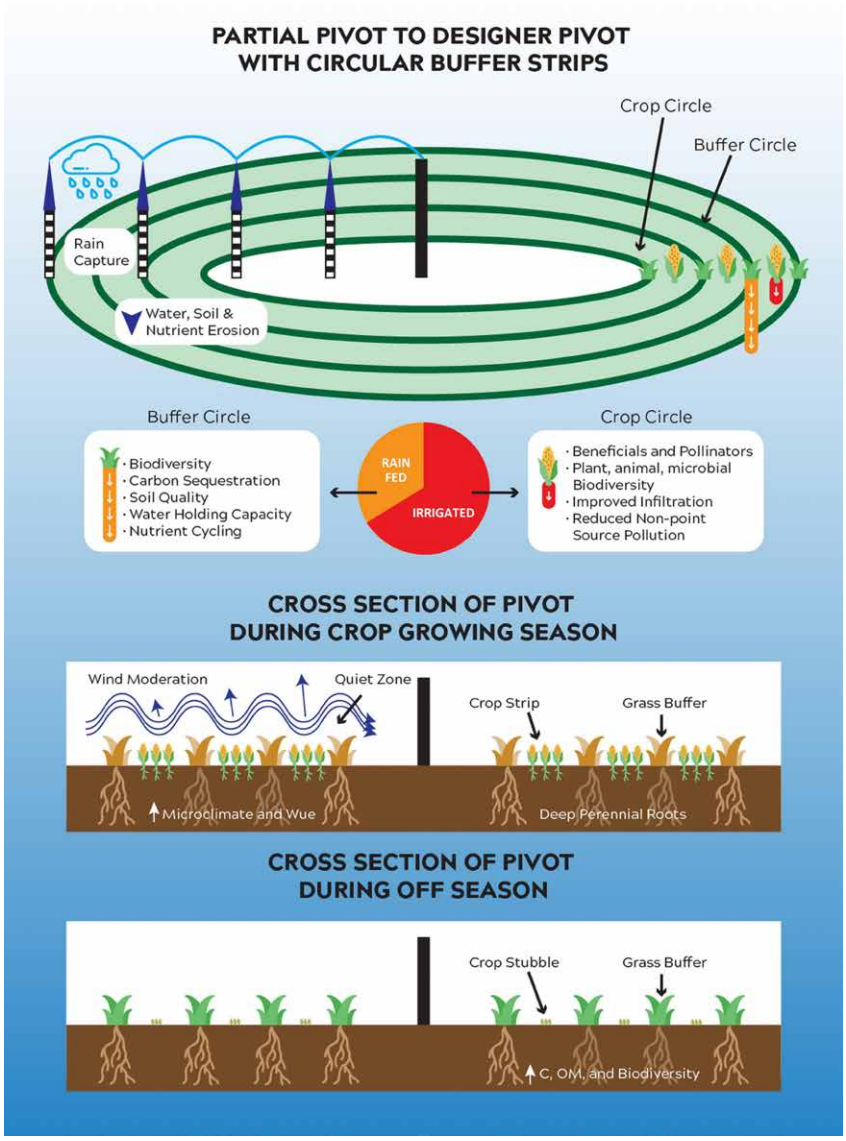
This is a simple, elegant solution with several advantages. Planting the buffer strips in circles ensures that part of the strip will always be at a right angle to the direction of the wind, protecting the crop strips from wind and sand abrasion regardless of any changes in direction and reducing water evaporation from soil surface wetted frequently by centre pivot irrigation systems. It also fits in with the widely used circular pivot field design. Using multiple buffer strip circles is expected to increase their protective effect, and this approach is easier to deploy and manage than the alternative of planting tall trees as a windbreak around the pivots’ outer edges.

In addition to reducing water consumption, the team expects that there will be further, indirect benefits to agricultural productivity, and to the surrounding environment. The buffer strips will help to prevent the runoff of water, decreasing soil erosion and retaining nutrients within the system. The system will work efficiently even if wind direction and slope of water runoff are different. These nutrients will remain available for the crops at a later stage and reduce the need for fertilisers. The team also predict that the organic matter content of the soil will also improve.

Similar to natural ecosystems, the presence of native grasses and forbs is likely to support insect predators, which will reduce pest numbers, decreasing the levels of pesticides needed. In turn, reduced pesticide together with increased habitat and forage, will boost pollinator numbers and increase the local biodiversity. Due to addition of perennials into annual system soil microbial, bird and animal diversity is also expected to increase. The buffer strips will also benefit the environment through preventing the pollution of surrounding water bodies with fertilisers and pesticides that would otherwise be carried in runoff.

The environmental advantages of planting with perennial grasses in concentric circles extend beyond the local scale: as well as absorbing atmospheric carbon dioxide – the key driver behind climate change – they can absorb nitrogen compounds that are also harmful to the environment. The system is also expected to be more energy-efficient, as it reduces the need to pump irrigation water, perform tillage and provide inputs.

Dr Angadi’s team’s CBS approach compares favourably to other mechanisms of reducing wind effects and evaporation losses, such as the relatively expensive drip-irrigation system. Tillage to increase soil surface roughness, or planting with herbicide-terminated wheat for the protection effects of its stubble, require higher inputs of water



and energy. In comparison to corn, buffer strips of perennial grasses require little maintenance and relatively low amounts of fertiliser, pesticides and irrigation water. The grasses have a longer growing season, which maximises their ability to modulate the field's microclimate and absorb carbon dioxide. Finally, grass buffer strips could also be used for livestock forage, as part of an integrated farming system.

Exploring the System's Potential

Dr Angadi and his colleagues are carrying out a series of preliminary experiments to optimise their design, supported by a USDA-NIFA seed grant. 'There is a need to assess linkages between the Food-Energy-Water components of CBS and develop quantitative relationships to advance this technology to large scale adoption in the Southern Great Plains,' they explain in their proposal.

To make this happen, the team will assess the performance of the CBS system from multiple perspectives, measuring its effects on productivity, profitability and the environment. They are hoping to identify the most suitable width, species composition, and number of buffer strips. The grass strips must be wide enough to offer the potential benefits, but not so wide as to greatly restrict the area available to the main crop, which is often corn.

Another important factor is the size of the equipment that will be needed to maintain the plot. Farm operations on the circular strips, including planting, have become possible in recent decades thanks to the development of RTK-GPS guiding systems, which are increasingly common in the Southern Great Plains. Pivots attached to lower-yielding irrigation wells would be suited to wider buffer strips.

The team plans to use tall native grasses and a few native forbs, with the outermost buffer ring composed of tall perennials to modulate the harsher conditions. The grasses must be selected carefully so as not to compete for resources with the main crop. One of the challenges that Dr Angadi and his team anticipate is that the design may complicate the process of aerial spraying. However, other processes may be easier – for example, farm maintenance vehicles can drive on the buffer strips to access the centre pivot.

The team is currently conducting the trial at NMSU Agricultural Science Centre at Clovis (which can be seen in the satellite image at 34.598, −103.217) using corn as the main crop, and comparing pivots with and without buffer strips. They are monitoring a suite of environmental parameters, using temperature and humidity sensors, anemometers to measure wind speed, and lysimeters to measure soil evaporation. These measurements will allow the team to quantify the modulatory effect on wind and microclimate of using different numbers of strips, and varying their positions, as well as the time taken for the effects to become apparent. They will also assess differences in the biomass of corn harvested, the yield of grass forage, the changes in soil quality, and the energy inputs to the system, as well as surveying the insects on the plots to understand biological pest control services.

Once the team has fine-tuned the system's design, it can be tailored to suit a range of conditions, depending on the needs of individual farmers, and the particular climate. The idea has already gained the support of local stakeholders, including the major regional centre pivot irrigation company and a grass seed company. Farmers are also willing to try it out, as one remarked: 'Show us the benefits, and we will make it work with our equipment'.

The team's CBS approach has the potential to help ensure the long-term sustainability of agriculture in the Southern Great Plains, in line with the four principles of the National Academies of Sciences' future farming strategy: improving the productivity of crops, the condition of the natural environment, agricultural profitability, and the quality of life for agricultural communities. Dr Angadi and his team are excited about its prospects, and believe that its effects on productivity and ecosystem services will only increase over the longer-term.

Meet the researcher

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Dr Sangu Angadi obtained his BSc in Agronomy from the University of Agricultural Sciences in Bangalore, India, in 1983, followed by an MSc in 1985. He then spent two years as Rural Development Officer at the State Bank of Saurashtra in Gujarat, before becoming Assistant Professor at the University of Agricultural Sciences in Dharwad. Between 1992 and 1998 he worked as Graduate Research and Teaching Assistant at the Department of Plant Science of the University of Manitoba, Winnipeg, Canada. From 1998 to 2003, he worked in Semiarid Prairie Agriculture Research Center of Agriculture Canada at Swift Current, Saskatchewan as a Contract Scientist. In 2001, Dr Angadi was awarded a PhD in Plant Sciences from the University of Manitoba, where he worked as a Faculty Research Associate from 2003 to 2005. He spent two months as a statistician at the Canadian Government's Grain Commission before accepting his current position of Associate Professor at the Department of Plant and Environmental Sciences and Agricultural Science Center at New Mexico State University, where he is currently based.

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BIOCHAR AND SOIL DYNAMICS

Renewable biofuels are a carbon-neutral alternative to fossil fuels, but they have their own complications. One problem is that growing and harvesting crops for biofuel depletes soil of valuable nutrients. To mitigate this, a by-product of making biofuel – known as biochar – can be added back into the soil to sustain its quality. **Dr Dedrick Davis** at Alabama A&M University has set out to determine how biochar might affect soil's physical properties, especially with regards to the movement of heat and water.

Coupled Heat and Water Transfer

Topsoil plays a far more crucial role in agriculture and the environment than most people might think. Even though topsoil holds little of the Earth's water supply, it acts as an important boundary between the ground and our atmosphere. As such, processes going on in soil can have far-reaching effects.

One of those processes is the movement of heat and water through soil, which is the focus of Dr Dedrick Davis' work at Alabama A&M University. 'The heat and water transfer processes are intertwined and scientists refer to this process as coupled heat and water transfer,' Dr Davis explains.

The coupled transfer of heat and water in soil is driven by fluctuating temperature and radiation throughout the course of our 24-hour day. During the night, the soil's surface cools and causes heat to transfer upwards into the air. Deeper, cooler water also moves up with this heat and rewets the soil. In the morning, the sun starts to warm the ground, vaporising the water on top of the soil, and this evaporation also releases heat into the atmosphere. 'As a result of the temperature gradients that develop in response to heat transfer, water transfer occurs in both the liquid and vapor phases,' explains Dr Davis.

This cycle controls things going on near the soil surface. In a paper published in the *Soil Science Society of America Journal* in 2014, Dr Davis and his colleagues explain the importance of these processes: 'The temperature fluctuations and water contents that result from the coupled transfer of heat and water in soil are important for all physical, biological, and chemical processes that occur near the soil surface.'
There are many examples of these processes. For instance, the amount of water present in soil and the soil temperature affects microbial activity, which is important for breaking down organic material. Microbes are also known to degrade pesticides. Other examples are seed germination and plant growth, which heavily depend on both soil temperature and moisture.

New Ways to Measure Soil Properties

Many factors influence the movement of heat and water through soil, thereby affecting rates of evaporation near the surface. For example, Dr Davis and his colleagues have found that a soil's wettability – the ability of a soil to take in water – can affect how heat and water will move through it.

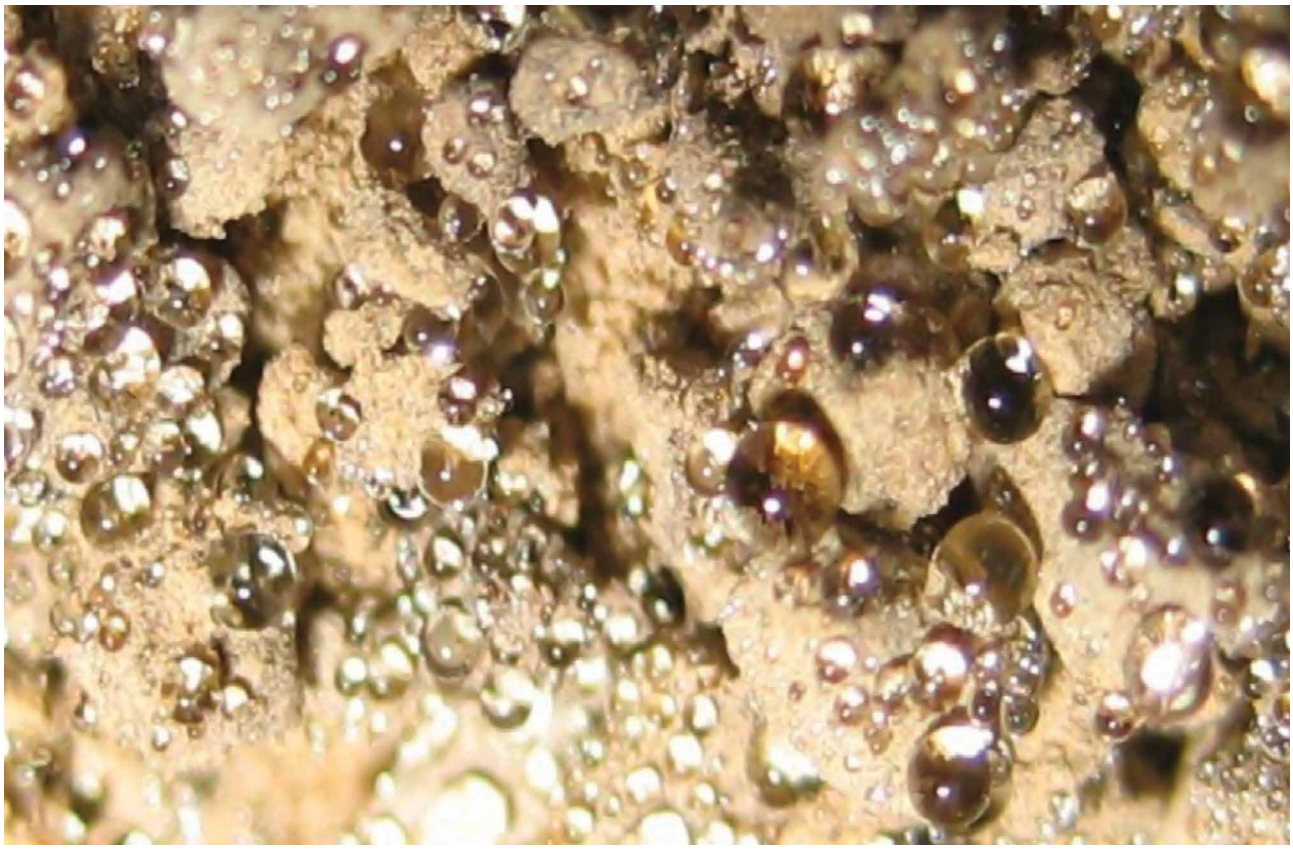
To figure this out, the researchers used heat-pulse sensors to measure soil temperature, water content, thermal conductivity and thermal diffusivity in 'closed soil cells'. These cells contained sand and silt loam soils that were either in their natural, wettable, state or in an artificially-induced hydrophobic (or water-repellent) state. To make these soils become hydrophobic, the scientists added dichlorodimethylsilane (DCDMS) – a chemical agent often used to create coatings that repel water and prevent it from being absorbed into a material.

Using heat exchangers, the team applied temperature gradients to each of the soil cells for 28 days. Heat-pulse sensors that they placed at different depths measured non-linear temperature distributions in response to the temperature gradients applied to both the wettable and hydrophobic soils. However, with regards to water transfer,

the two types of soil did not behave the same. Using these sensors, the team was able to measure that water transfer in the water-repellent silt loam was reduced by 56% compared with its wettable counterparts. This led the scientists to conclude that water vapour transfer would thereby be lower as well.

Their findings, which they published in 2014 in the *Soil Science Society of America Journal*, have real-life implications. Water repellency in soils can affect how well plants grow or how quickly water can infiltrate into soil. In the latter case, probably the best-known example

‘The project will enhance ongoing research in biochar amended soils and will help to further establish a fundamental basis for the use of biochar as a soil amendment and as a potential tool for climate change mitigation.’



is a flash flood, which is triggered due to the retardation of water infiltration in a soil such as desert sands after a hard rain. Another important implication for Dr Davis is that the processes occurring near the soil surface – such as soil water evaporation – are also altered.

This study was also important in that it honed the methodology and experimental setup he would use in future work funded by the USDA National Institute of Food and Agriculture Evans-Allen program. This program, established in 1977, provides funding for food and agricultural research at 1890 land-grant institutions and aims to improve stewardship of natural resources and improve crop diversity, among other goals.

Biochar – How Does It Really Affect Soil?

Since changing a soil’s characteristics can alter the coupled transfer of heat and water, agriculturalists need to be careful about any alterations that they may make to the soil or amendments that they may apply to the soil. One such soil amendment that captured Dr Davis’ attention was biochar, the focus of his Evans-Allen proposal.

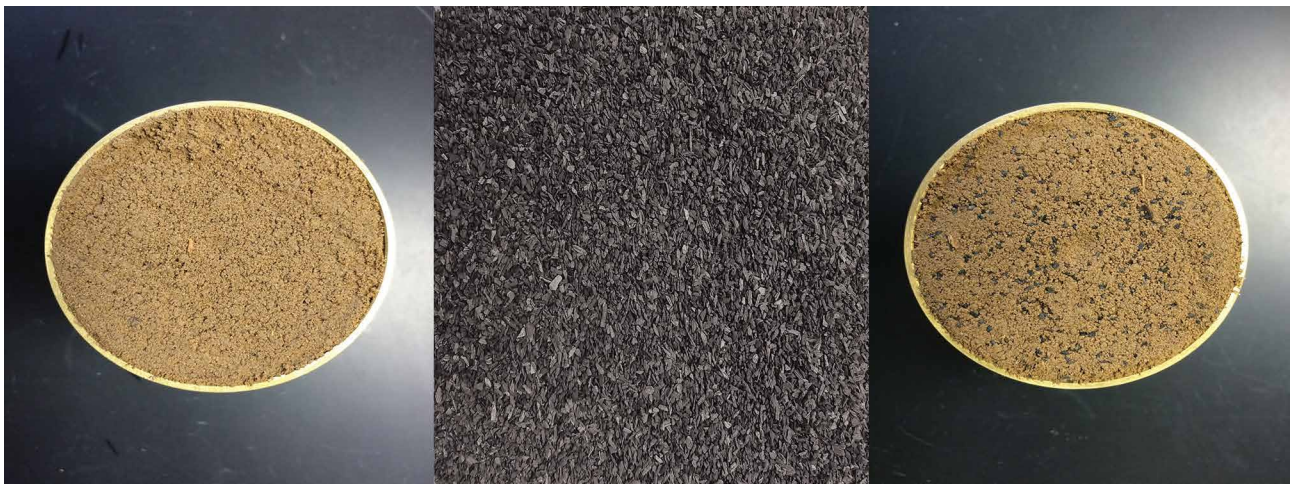
Biochar is a by-product of pyrolysis, a process used in the production of biofuels. During pyrolysis, any number of types of organic or plant dry biomass (also known as ‘lignocelluloses’) – such as weeds, plants, lumber waste, crop residues and manure – is heated in the absence of oxygen. Pyrolysis basically speeds up the decomposition of biomass and produces gases (known as syngas) and bio-oils (used for fuel), along with biochar.

Biochar is a rich, black and charcoal-like substance – hence the name, which is derived from the words biomass and charcoal. This by-product can increase the retention of nutrients in soil and improve the soil’s water holding ability.

Biochar has also been touted as important for reducing carbon dioxide in the atmosphere, and thus mitigating climate change. This is firstly because biofuel crops absorb carbon dioxide as they grow, and when biochar is created during the processing of these crops, carbon in the biomass is transformed into more stable forms. If added to soil, this carbon can remain captured in the soil for thousands of years. Secondly, reducing our reliance on fossil fuels (carbon positive) by replacing them with biofuels (carbon neutral) will help to reduce our carbon dioxide emissions. Therefore, if adding biochar to soil solves some of the problems associated with growing biofuel crops, it will play a dual-role in mitigating climate change.

Despite these benefits, little is known about how biochar might affect the coupled movement of heat and water in soil, and this is the current focus of Dr Davis’ research.

Dr Davis first worked with biochar on a collaborative project at Iowa State University. ‘I was responsible for making soil physical property measurements such as bulk density,



saturated hydraulic conductivity, surface area, and soil water retention,' he tells us. 'This study was the first in which I worked with biochar and where my interest in biochar began.'

Today, his ongoing project has four components, or objectives, in determining how biochar affects soil. First, he wants to measure how biochar affects the ability of soils to retain water. As mentioned earlier, the amount of water that soil can hold is a crucial factor for processes such as crop growth. Dr Davis hypothesises that biochar increases the amount of water that can be retained.

Secondly, Dr Davis and his team aim to determine how biochar affects soil thermal properties and heat transfer in soil. The third objective is to measure water and temperature redistribution in soils that have been amended by biochar. The fourth and final component is to see how biochar affects soil water evaporation. In all of these experiments, the researchers will use different soil types to see how biochar changes their properties.

Dr Davis hopes that the Evans-Allen project will help increase our understanding of using biochar, towards efforts to mitigate climate change.

Next Steps

'The next steps for my work are to extend it to the field,' says Dr Davis. He explains that most studies on the impact of biochar on soil have been limited to the laboratory. Therefore, its impact on heat and water transfer near the soil surface in a real-world setting is not fully understood.

For the next phase of his research, Dr Davis will evaluate the impact of biochar on heat and water movement near the soil surface in the field. In this project, they will use the expertise and methods they have been building on for the last few years in the laboratory. For their study site, they have chosen the Winfred Thomas Agricultural Research Station in Haze Green, Alabama. The station is located in the northern part of Alabama in the Tennessee Valley region – a significant crop and poultry growing area. They hope to begin their experiments here in the spring of 2017.

For these plot scale experiments, Dr Davis will make near surface measurements of soil water content and soil temperature. Each plot will be given a slightly different treatment. For each plot, different

amounts of biochar will be applied and incorporated to a depth of 15 cm. All plots will initially be planted to soybeans and rotated with corn.

In addition to measuring soil water content and temperature near the soil surface, Dr Davis will also make measurements of time variable soil physical and hydraulic properties such as bulk density and soil water infiltration. The results of this significant field study should increase our understanding of how biochar affects soil physical properties and soil physical quality. Dr Davis emphasises that the 'project will enhance ongoing research in biochar amended soils and will help to further establish a fundamental basis for the use of biochar as a soil amendment and as a potential tool for climate change mitigation.'

Meet the researcher

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Dr Dedrick Davis is currently Assistant Professor of Soil Physics at Alabama A&M University, where he conducts research, advises graduate students and teaches courses such as soil physics and scientific writing. In 2012, Dr Davis received his PhD in Soil Science and Environmental Science from Iowa State University, after obtaining his MS degree from there in 2005. His research focuses on measuring and characterising soil physical properties and quantifying soil physical processes using field experiments, laboratory experiments, and novel measurement techniques. Specifically, Dr Davis is interested in coupled heat and water transfer and soil water retention in soil. He is a member of the Soil Science Society of America and the American Society of Agronomy, among others, and participates in several outreach programs that encourage the participation of underrepresented students in STEM.

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ALABAMA A&M UNIVERSITY

CLIMATE AND AGRICULTURAL RESILIENCE IN THE AMERICAN SOUTHWEST: SAFEGUARDING ANCIENT TRADITIONS AGAINST MODERN THREATS

Sustaining the availability of clean water is one of the greatest challenges humanity will face, as climate change leads to hotter, more extreme weather patterns that disrupt normal snow and rainfall worldwide. These changes are predicted to hit arid climates particularly hard, and the effects in such areas are already being felt in many countries across the globe. **Dr Maureen McCarthy** at the University of Nevada and the Desert Research Institute is working with Native American communities to help promote and preserve ancient tribal agricultural practices and water use in the United States.

Global climate change is likely to be the paramount challenge facing humanity over the next hundred years. Driven by human-induced disruptions to the carbon cycle, largely due to burning fossil fuels and worldwide deforestation, climate change threatens to drastically alter our way of life through reduced water availability, widespread food insecurity, and extreme weather events. These effects are expected to hit certain communities particularly hard, and those that rely on agriculture in arid climates are predicted to be some of the most affected.

Native American reservations in the United States are largely relegated to arid climate zones with limited access to water resources. Many of these communities are heavily dependent on water from rain and snowpack to support rangeland livestock and dryland, and irrigated farming of crops for traditional uses and trade. Dr Maureen McCarthy leads two multimillion-dollar research projects with two goals: studying the effects of climate change as they unfurl, and working with Native American communities to leverage both native wisdom and western science to enhance tribal agricultural resilience. Resilience is defined as the ability of communities to adapt to more frequent droughts and extreme weather events that go hand in hand with warming temperatures.

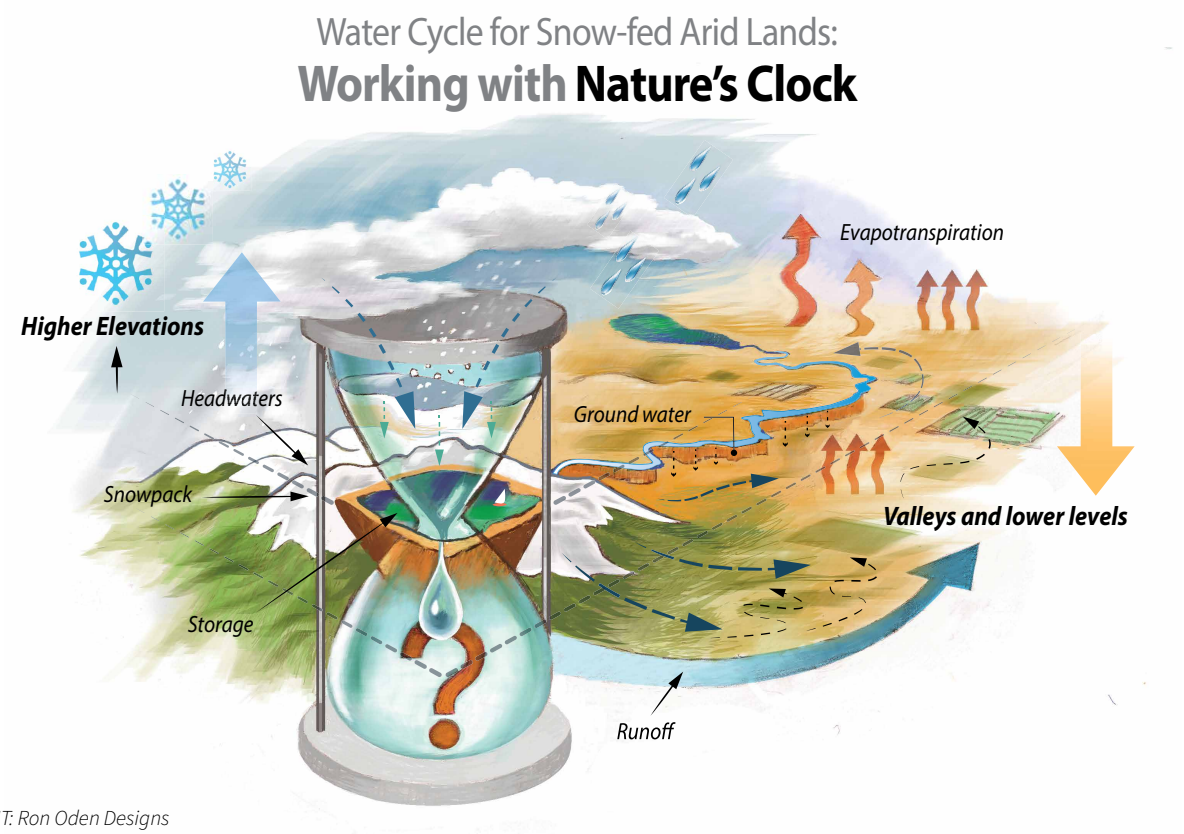
Her team's 'Native Water on Arid Lands' project focuses on tribal reservations in the American Southwest and Great Basin – areas that are normally subject to dry climatic conditions. These areas are likely to be hit hard by reduced water availability as temperatures warm and annual snowpacks in the surrounding mountains decline. Her other project, entitled 'Water for the Seasons', models climate impacts on water sustainability in a representative snow-fed arid land river system with competing demands from urban communities, agricultural producers, and tribal communities dependent on river flows for fish and crops.

Throughout the Western United States, water is primarily sourced from snowpack, which accumulates in winter and gradually melts to feed irrigated agricultural lands, urban communities, and energy production throughout the dry season. As global temperatures rise, this critical cycle is becoming disrupted as more winter precipitation falls as rain (not snow) in the mountains, decreasing nature's vast reservoir of water needed to support crops, livestock,

and people throughout the dry season. In addition, hotter summers cause more water to evaporate from rivers, streams and plants, decreasing water supplies and increasing demand.

Indeed, increasing temperatures are disrupting nature's clock at an unprecedented pace, resulting in shorter winters, longer summers, earlier springs, later falls, and challenging both rural and urban societies' ability to adapt. Punctuating these changes are more frequent extreme storms and floods and longer droughts, which further degrade community resilience. These challenges are not unique to the Western United States given that over 50% of the world's population relies on snow from either annual snowpack or glacier runoff as their primary source of water.

Both of Dr Maureen McCarthy's projects involve partnerships with tribal communities – joining western science and traditional knowledge



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to enhance tribal agriculture and create opportunities to educate and promote the careers of Native American students and researchers, while simultaneously respecting the agricultural, ecological and cultural integrity of American Indian communities. Many of the tribal members she engages with have a deep appreciation of the need for sustainable agriculture and land-use practices, and are eager to tie ancient traditions to modern conservation science. As Dr McCarthy's colleague, Dr Karletta Chief (member of the Navajo Nation and Associate Professor at the University of Arizona), explains: 'Indigenous peoples in North America have a long history of understanding their societies as having an intimate relationship with their physical environments. Their cultures, traditions, and identities are integrally linked to the land, water and sacred places of their homelands.'

Dr McCarthy feels fortunate to work with Native American tribes, whose long legacy of living in harmony with the land imbues innate understanding of human adaptation to ecological and climatic change. 'It was very humbling and restorative when I began working with the Native American communities,' she says, 'to realise that native wisdom complements and strengthens western science and both are needed to understand the impacts of climatic change on water and agriculture for millennia to come. Community resilience derives from both traditional and scientific sources of knowledge.'

Winter Snows and Summer Crops

Having started in 2014, 'Water for the Seasons' (WftS) is a multi-year research project working with tribal and non-tribal water, agriculture, and natural resource managers in the Truckee-Carson River System (TCRS) of Northern California and Nevada. Project goals are to develop an integrated suite of hydroclimatic and socioeconomic models to assess the climate resiliency of the TCRS and to understand how this approach can be scaled or transferred to snow-fed arid systems around the world.

Urban, rural and tribal communities along the Truckee and Carson Rivers in Nevada are located in the arid lands of the Great Basin in the State of Nevada. Nevada is the driest state in the nation, receiving less than 190 mm of rainfall annually with northern Nevada depending entirely on water from melting snowpack in the Sierra Nevada Mountains. The TCRS is representative of water challenges throughout the Western United States with a compact geography that is only 193 km from headwaters to terminus. Competing demands for water originate from urban growth, industrial development, rural agriculture, and measures to protect endangered fish species and other natural resources.

Communities in this region rely on accumulated snow in the Sierra Nevada mountain range that melt in spring to feed rivers, streams, reservoirs, and aquifers that, in turn, provide water throughout the dry summer and fall seasons. These areas are facing increasing challenges, as milder winters mean less snow to feed the spring runoff and more winter floods, which arrive too early for agricultural production or to be captured by reservoir operations.

Dr McCarthy and her colleagues are developing climate scenarios informed by both scientists and stakeholders, using these scenarios to model water availability, integrating the hydroclimatic models with socioeconomic models of human behaviour to better understand system resiliency, and finally, assessing how well this approach transfers to other snow-fed arid landscapes around the world.

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Thus far, the team has been successful in building models that incorporate insight about how diverse communities – urban planners, rural farmers and ranchers, Native American fisherman and farmers, and natural resource managers – respond to changes in water supplies due to droughts, warming temperatures, and increased inter-annual variability it precipitation. They have developed a suite of scenarios based on historical data, paleoclimatic records, and global climate models to probe the sustainability of water supplies in a future of longer droughts punctuated by extremely warm, wet seasons. Water availability in the TCRS depends on snowpack accumulation, river basin geography/topography, groundwater/surface-water interactions, and reservoir operations governed by complex legal doctrines for allocating water to water right holders and maintaining stream flows to protect endangered fish. With these data, the researchers and stakeholders can estimate inter-annual water availability and assess alternative water management, agriculture, and urban development strategies to enhance community resilience in a changing climate.

With these models in hand, WftS researchers hold regular workshops with stakeholders from the three tribes (Washoe, Pyramid Lake Pauite, and Fallon-Shoshone Paiute) and other water users and managers, presenting their results and taking feedback on alternative water management strategies. These meetings help the various parties understand one another’s water usage and needs, while providing valuable information to the research team about what management strategies might be socially, economically, and culturally practical. They also help the team better understand the times of year when communities are most vulnerable to water stress, and how changes in water management during these periods can build more climate resilient systems.

Thanks to the WftS team, the Truckee Meadows Water Authority is developing a new Drought Contingency Plan incorporating their data with economic models to help ensure water security for the growing urban areas. Further, the team’s techniques are being used to build regional climate scenarios for the American Southwest to predict areas of sociocultural, ecological, and infrastructure vulnerabilities due to climate change.

WftS researchers are collecting data in the Carson Valley on the viability of strategies such as managed aquifer recharge, which replenishes groundwater by directing excess water to dormant agricultural fields during warm winter storms that can, in turn, be pumped for irrigation during the growing season. The team plans to use this data to assess alternative crop and irrigation practices that can both increase surface water use efficiency and replenish groundwater supplies. They are also working to understand the economic drivers for land and water management decision-making and the willingness of various stakeholders to participate in conservation programs and water sharing economies.

Finally, the researchers are investigating how the insights gleaned from this region are applicable to other snow-fed arid regions around the world, thus testing the applicability and transferability of this approach to help other communities facing water sustainability challenges due to climate change. As the WftS project transitions into its final years, Dr McCarthy and her team are continuing to build on these successes and share knowledge about water sustainability and climate resilience with the scientific community, urban water managers, natural resource managers, and rural farmers and ranchers.

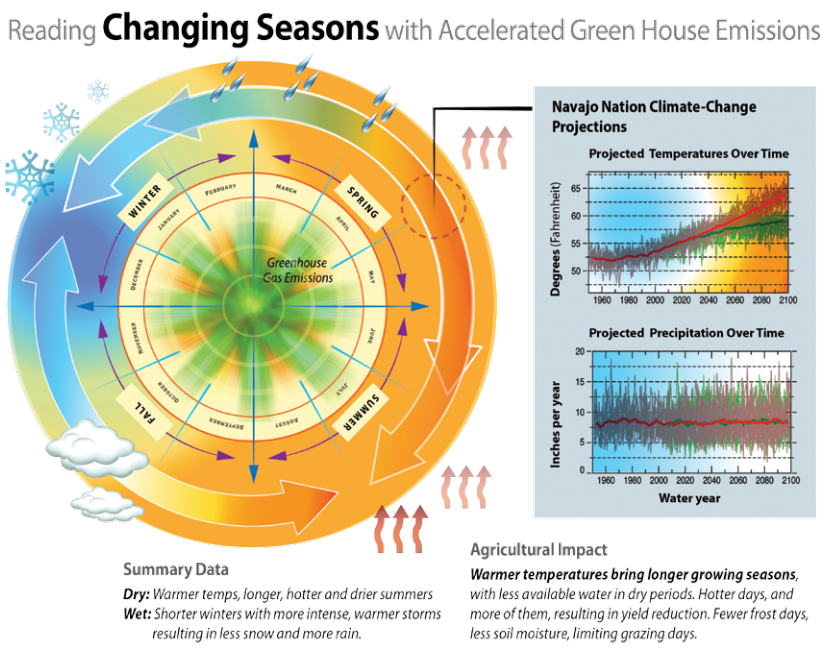
Native Waters in the Desert

Indigenous communities in North America have ancestral ties to the land that span thousands of years, rooted in agrarian cultural and spiritual practices intimately associated with the health and wellbeing of terrestrial and aquatic natural resources. The world view of Native American communities is ecosystem- and watershed-based, inextricably bound to the land and supporting a holistic approach to living in balance with the drivers of change in air, land and water.

American Indian land tenure and water rights laws enacted by the US Government during the 19th and 20th centuries forced Native Americans to either relocate or consolidate on designated reservations. It also fractionated tribal land ownership and mandated conversion of traditional agriculture to Western agrarian practices.

In the American Southwest and Great Basin Desert, Native Americans lived on expansive ancestral lands for thousands of years before European settlement, sustaining themselves through a combination of hunting, fishing, gathering seeds and plants, raising livestock and crops, and trading with neighbouring communities. Sustaining these agricultural practices has become increasingly difficult in recent years due to rapid changes in climate and the growing demands for water and land by the surrounding non-tribal communities. In addition, most Native American communities face daunting socioeconomic challenges including high levels of poverty and disease that influence land-use decision making by tribal governments.

‘Native Water on Arid Lands’ (NWAL) is a multimillion dollar five-year project involving diverse tribal partners from reservations across the largely arid lands of the American Southwest. NWAL’s primary goal is to bring scientists together with tribal farmers, ranchers, resource managers, educators, and council members, to assess the impacts of climate change on water resources and to enhance the resiliency of traditional and trade agricultural practices on Native American reservations.



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Dr McCarthy and her colleagues are accomplishing this goal through researching barriers and opportunities to improve the availability, accessibility and use of water for agriculture through a combination of traditional knowledge, altered crop and livestock practices, and water trading agreements. Through a partnership with FALCON – a network of 37 Tribal Colleges & Universities in the United States – the NWAL team is building capacity and creating educational opportunities for tribal members, thus supporting the next generation of tribal leaders that could shape policy and practice for water and agriculture reservations.

Native American researchers and outreach educators on the NWAL team are essential to creating cultural bridges between the scientific and tribal communities to share and co-produce the knowledge needed to enhance the long-term health and productivity of Native American agriculture.

NWAL researchers are evaluating how tribal water rights impact water availability and usage for sustenance and trade agriculture. They are also using global climate model data to assess the impacts of warming temperatures on water supplies at the river basin and watershed scales. The team has provided the tribes participating in the study with reservation-specific downscaled projections of future temperature and precipitation patterns under different carbon emission scenarios. These data are used to estimate changes in growing season timing and duration, minimum/maximum temperatures, snow vs. rainfall profiles, and precipitation timing and intensity.

Since 2015, the NWAL team has been hosting an annual Tribal Summit, where scientists and Native American farmers, ranchers, resource managers, and community leaders convene to discuss traditional and production agriculture practices, water challenges and developments and to share knowledge about community and agricultural resilience. The summit features workshops and panel sessions devoted to agricultural resilience issues, such as innovative water trading arrangements, rangeland conservation practices, groundwater/surface-water management, and traditional knowledge of seeds and cultivation practices that enhance drought resilience.

Faculty members and students from the Tribal Colleges and Universities are partnered with NWAL researchers to develop agricultural research projects on reservations. For example, faculty members at Aaniiih Nakoda College (ANC) in Montana are working with an agricultural ecologist from the Desert Research Institute to evaluate the effectiveness and efficiency of different irrigation practices used in the ANC community garden. Expanding projects like these not only help to inform water and agriculture policies, but also help to recruit and train a new generation of Native American researchers and resource managers.

A critical component of sustaining agricultural activity on tribal reservations is working with non-tribal state and local organisations to develop water policies that respect current and future Native American community needs. NWAL researchers are evaluating current agricultural water rights policies and identifying policies that may present barriers to climate resilience for tribal agriculture and water availability. These are used to develop guidelines for collaborative agreements between tribes, other water users, and public agencies. Through its collaboration with the Tribal Colleges, NWAL researchers are conducting research that integrates indigenous and western agricultural practices to diversify and enhance crop production, sustain water supplies, improve soil health, and sequester carbon. These activities provide education and outreach opportunities to support water, agriculture, and climate resiliency planning with tribal communities.

Sustainable and equitable water use in arid land agriculture will be critical to the survival of Native Americans as climate change progresses, and Dr McCarthy's NWAL project is dedicated to ensuring Native communities have access to a wide range of native and western data and knowledge that can help protect their cultures and their traditions for the millennium to come.

Protecting the Future of Tribal Lands

Water scarcity and security are likely to continue presenting challenges as global climate change progresses. By focusing on developing sustainable water use among some of the world's most vulnerable climates and populations, researchers like Dr McCarthy help inform models, policies and land management practices with far-reaching applications. Programs like WftS and NWAL not only help to protect tribal lands and traditions, but also illuminate paths forward for sustainable water management in communities worldwide.

As Dr McCarthy describes: 'Working with our tribal community partners and leveraging the knowledge gained from WftS and NWAL, we are bringing to bear the combined power of traditional knowledge and western science to strengthen Native American food and water security and improve community wellbeing.'

Meet the researcher

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Dr Maureen McCarthy began her research career with a PhD in Chemical Physics from the University of Colorado. She spent years serving in senior positions in the United States Government national security community in the Departments of Defense, Energy and Homeland Security, and participated in the first National Intelligence Assessment on the National Security Implications of Climate Change in 2008. Her realisation that climate change poses the most critical issue of our time underlies her current focus on climate impacts to both national security and community resilience, with the aim of building bridges between scientists and the people most affected. In 2009, she left Washington, D.C. to move west and subsequently joined the faculty at the University of Nevada, Reno and the Desert Research Institute directing major research programs in Lake Tahoe and the Great Basin. Dr McCarthy leads multidisciplinary teams of researchers focused on understanding the impacts of climatic change on tribal and non-tribal communities in the American Southwest, and evaluating practices policies to enhance agriculture resilience and water sustainability.

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CROPS FOR A CHANGING WORLD

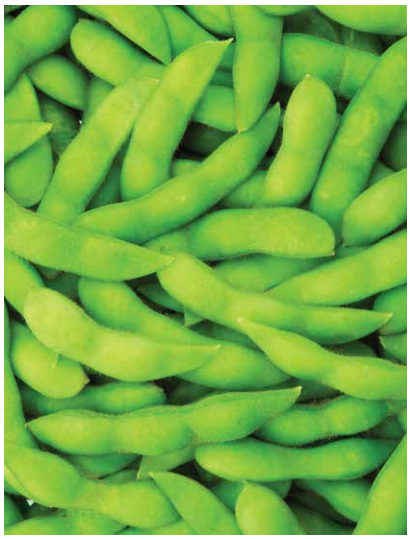
Professor Robert Aiken, Professor Vara Prasad and Professor Alan Schlegel at Kansas State University develop new strategies to cultivate some of the world's most important food crops, in order to adapt our agricultural systems to the effects of climate change.

Climate change is expected to dramatically alter global weather patterns, and increase the variability in conditions. This will have detrimental knock-on effects for activities that depend on the climate, such as crop cultivation. Every crop requires its own particular growing conditions, and any change in these could affect productivity. Without appropriate adjustments to cultivation methods, there are concerns that the likely decrease in yield could endanger global food security. The expected changes include higher levels of carbon dioxide, higher temperatures and greater variation in rainfall. Maximising crop yields under these new conditions will require innovative solutions, based on understanding the mechanisms that limit productivity under different climatic conditions.

In this article, we explore the work of three scientists at the forefront of research into the effect of extreme and changing environmental conditions on crop cultivation. Professor Robert Aiken, Professor Vara Prasad and Professor Alan Schlegel work with some of the world's most important food crops – wheat, rice, corn, soybean and sorghum – to develop new strategies for cultivation, which will be vital in the process of adapting agricultural systems to the impacts of climate change.

Their research includes assessing the effects of an array of environmental and management conditions that can affect crop productivity, including increased temperature and carbon dioxide levels, water shortages and different crop rotation strategies, as well as understanding the mechanisms through which these conditions impact crop physiology. Their base at Kansas State University provides the opportunity to conduct field experiments in a state with the largest wheat production in the US. With 40% of Kansas's wheat area semi-arid, it is a particularly suitable location to study mechanisms for improving crop tolerance to drought and heat.

Robert Aiken is Associate Professor and Research Crop Scientist, and focuses on developing management strategies to improve crop tolerance to stress caused by cold, heat or water deficits in semi-arid climates. Vara Prasad, University Distinguished Professor of Crop Ecophysiology, is the director of the Feed the Future Innovation Lab for Collaborative Research on Sustainable Intensification. He is also interested in the mechanisms of crop stress responses, and in developing sustainable crop systems. Alan Schegel, Professor of Agronomy, researches nutrient and water management approaches in dryland and irrigated systems, to identify routes to more efficient cultivation strategies. Their individual research projects interact and inform each other, building on Professors Aiken, Prasad and Schegel's shared expertise in agricultural biology and management, and facilitating their progress in developing resilient approaches to cultivation.



TOWARDS DROUGHT- AND HEAT-TOLERANT WHEAT

With rising global temperatures, incidences of drought are predicted to become increasingly common, posing challenges to the cultivation of many staple crops, including wheat. **Professor Robert Aiken** is exploring ways to improve crop resilience in the face of these effects.

Crop Rotations to Combat the Effects of Drought

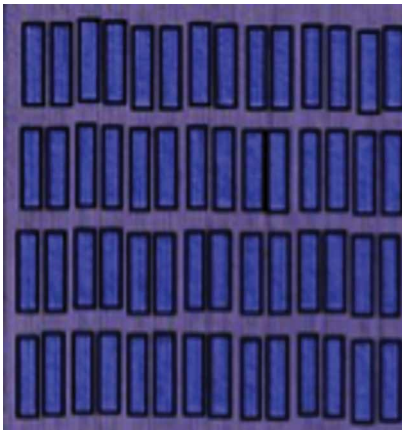
Climate change is expected to lead to higher temperatures and greater variations in weather, which will also increase the risk of drought. Even under current conditions, the supply of water available to crops in semi-arid regions can limit their productivity, and yields in wetter regions can be impacted by drought. The effects of climate change on drought risk are compounded by the constant increase in the global demand for fresh water. Improving the productivity of crops in water-limited regions is essential to meet the world's food requirements against the backdrop of increasingly unpredictable weather conditions.

Regional water availability is also one of the factors that limits the cultivation of wheat, a staple food crop in much of the world. One strategy to improve the efficiency of water use in crops such as wheat is to leave agricultural land fallow in between cropping, which can increase the rate at which the water stored in soil is replenished. However, this recharge is limited due to high losses caused by evaporation. To reduce these water losses in semi-arid regions, continuous crops can be substituted for the fallow period, which permits productive use of water due to plant transpiration. These fallow replacement crops for continuous cultivation can be cover crops, green manure crops or oilseed, grain, cereal or forage. The disadvantage of this approach is that fallow replacement crops can also reduce the soil water available to the following wheat crop, and green manure crops can decrease yields.

Improving wheat yields in water-limited areas could be achieved through understanding the influence of water use on the lower yields obtained in a continuous cropping approach, in relation to wheat grown after a fallow period. This could allow the intensification of cropping without an accompanying loss of productivity. Professor Robert Aiken's research focuses on identifying the factors regulating crop transpiration and its effect on yield in water-limited cropping systems.

Together with his colleagues at Kansas State University, he carried out an experiment to identify the effects of crop sequences and environmental variation on water use and yield in a winter wheat crop in a semi-arid part of Kansas. Between 2002 and 2008, the team cultivated winter wheat in 3-year crop rotations after a feed grain phase and an oilseed crop or fallow phase, under normal growing conditions and drought conditions. The results, published in 2013 in the *Agronomy Journal*, found that an oilseed replacement for fallow (spring canola, soybean or sunflower) had the greatest impact on wheat productivity. This reduced winter wheat biomass, grain yield and the corresponding net economic returns of sowing the wheat crop compared to wheat with fallow, by 18%, 31% and 56%, respectively. This was due to the impacts of the replacement crop on water availability for the wheat.

Under severe drought, the continuous crop had reduced grain yield. The water productivity, or yield relative to water input, was relatively low compared to previously recorded ranges. This suggests that the yield could be improved through management and genetic efforts such as using drought-adapted cultivars. The results indicate the benefits of continuous cropping should be weighed against the impact of reduced water available for subsequent wheat crops.



Identifying Drought-Tolerant Wheat Through Carbon Isotope Discrimination

Selective breeding of drought-tolerant wheat varieties is one of the most common approaches to improve the prospects of growing wheat in water-limited regions. Unfortunately, this has proven difficult to achieve in practice, because the relevant traits and their heritability are complex. An alternative method of identifying drought-adapted wheat varieties is through focussing on appropriate physiological traits with high heritability. It has been shown that higher plant transpiration efficiency is associated with lower values of a trait called Carbon Isotope Discrimination (CID), which is a measurement reflecting a process in photosynthesis using the enzyme Rubisco. Carbon dioxide assimilation with Rubisco uses a greater proportion of the C^{12} (the most common isotope of carbon) than C^{13} (a heavier form of carbon, containing an extra neutron).

Plant tissue thus has a higher ratio of C^{12} to C^{13} than the atmosphere, and the CID value is a measurement of this difference. If CID is a suitable indicator for drought tolerance, it should be correlated to grain yield in drought conditions. However, this association varies considerably across different studies, so it could be affected by differences in breeding lines and environmental conditions, such as the water availability.

To try to unravel these interactions, Professor Aiken and his colleagues carried out a study exploring the link between CID and yield in wheat exposed to drought conditions, using 58 different breeding lines in four field trials. Their results, published in *Euphytica* in 2015, found a significant positive correlation in three of the four trials. In the 43 preliminary breeding lines, there was a significant positive association in all three test environments. However, it was weaker in the advanced breeding lines, which were tested in one environment and suffered from winter injury. This may have affected the yield, and hence the CID/yield relationship.

The team also found a significant negative correlation between grain CID and protein content, which could reduce the utility of the wheat for baking. It is possible that this disadvantage could be mitigated by selecting for lines with higher nitrogen uptake and/or utilisation capacity, to improve protein production. Both sets of breeding lines had considerable genetic variation in grain CID, suggesting the potential for selective breeding for the trait. In three of the four environments, the scientists found that grain weight and CID showed a significant positive relationship. These results suggest that grain CID would be a suitable candidate trait to allow the selection of drought-resistant wheat varieties under moderate drought stress. As the positive association between CID and yield was more pronounced in the trial with post-flowering drought stress CID could be particularly relevant as a yield indicator for regions with dry spring conditions.

Canopy Imaging for Heat Tolerance

Wheat productivity in semi-arid regions, such as parts of Kansas, is also impacted by high temperatures. High temperatures affect pollination, seed development and canopy productivity, i.e. the plant biomass produced by the utilisation of light in photosynthesis. Like the variation of drought tolerance in wheat, some wheat species have higher heat tolerance. However, both conventional selective breeding approaches and genetic mapping methods require a large number of breeding lines to be assessed, which can be relatively time-consuming and expensive. Professor Aiken and his colleagues are currently testing a new method for detecting heat tolerance, which they hope could improve the speed of identifying heat-tolerant wheat lines. They are carrying out a two-year project in Kansas to explore the potential of predicting the heat tolerance of wheat lines from their canopy productivity. If this proves to be the case, they plan to test whether canopy imaging techniques could enable the rapid, high-throughput screening of wheat fields for heat tolerance. Such a tool would be a great asset to wheat breeders, allowing them to produce new, heat-tolerant wheat varieties, and improve agricultural resilience to high temperatures.

OPTIMISING YIELDS FOR CHANGING ENVIRONMENTS

In order to adapt to climate change, we need to understand the interactions of altered environmental conditions, including temperature and carbon dioxide levels. **Professor Vara Prasad's** work provides insights into these interactions, and explores management strategies for improving livelihoods in developing countries.

Elevated Temperatures Impact Rice Yield

Rice is another crop of huge global importance as a staple food. About 90% of rice cultivation is carried out in tropical regions in Asia, at temperatures close to the optimum for growth – around 30°C. Climate change is predicted to increase temperatures, and measurements in several tropical regions of Asia have shown increases in annual mean maximum and minimum temperatures. Rice yields are known to decline at higher temperatures in the growing season, and rice crops are particularly vulnerable to high temperature damage around the flowering period, as this impacts floret fertility and hence seed set. As rice is such an essential source of food for billions of people, it will be necessary to develop strategies to adapt rice cultivation to higher temperatures. Selective breeding approaches will require the identification of well-adapted varieties.

Professor Vara Prasad is an agronomist at Kansas State University, carrying out research into identifying optimal management strategies for food crops in changing environments. In an article published in 2006 in the journal *Field Crops Research*, Professor Prasad and his colleagues describe their study to identify differences in sensitivity to high temperatures between different rice species and cultivars, through the impacts on spikelet (flower) fertility and grain yield. They used temperature-controlled greenhouses in Gainesville, Florida, to investigate the effect of raising the environmental temperature from 28 to 33°C in 14 cultivars.

Their results showed that higher temperatures significantly decreased fertility and yield in all cases. The cultivar N-22 was the most tolerant, while others were considered either moderately or highly susceptible. However, there were no clear patterns across species (*Oryza sativa* or *glaberrima*), ecotype (*indica* or *japonica*), or origin (temperate or tropical). The main impacts of high temperature on spikelet fertility were through decreased pollen viability, pollen production and reception on the stigma – the female component of the flower. This led to a lower grain numbers and grain yield. Professor Prasad and his team suggest that the next steps for improving heat tolerance are the identification of relevant physiological or genetic traits, and selection for genotypes flowering at earlier times of the day, when temperatures are lower.

Interactions of Carbon Dioxide and Temperature in Sorghum

The effect of higher carbon dioxide levels on plant productivity involve many complex, interacting mechanisms. While increased carbon dioxide will negatively affect crop yields through higher global temperatures, it could also enhance plant photosynthetic rates and thus productivity. Despite this, studies of the interaction of elevated carbon dioxide and temperature levels on C3 crops (e.g., wheat, rice, soybean) have shown that the higher growth did not mitigate the damaging effects of high temperatures on yield.

C4 crops such as grain sorghum (*Sorghum bicolor*) are more tolerant of high temperature, and Professor Prasad and his colleagues authored one of the first systematic studies looking at the effects of higher carbon dioxide levels and temperature on their yield. Their results, published in 2006 in *Agricultural and Forest Metereology*, showed that the damaging effects of higher temperatures on pollen viability and grain

yield were increased at elevated carbon dioxide levels. In addition, while elevated carbon dioxide improved sorghum yield at lower temperatures, at higher temperatures it decreased yield. The authors suggest that the impacts of elevated carbon dioxide on fertility are due to its effect in increasing tissue temperatures. Stomatal sensitivity to carbon dioxide may differ between genotypes, which could allow the selection of cultivars with lower sensitivity and thus a smaller increase in leaf temperature. Similarly, genotypes may also vary in the response to floret fertility under high temperatures and can be used in breeding programs to enhance tolerance to higher temperature stress.

Advances in Heat Tolerance

Understanding the mechanisms of heat tolerance in crops is still a pressing issue. In a recent review article in *Field Crops Research in 2017*, Professor Prasad and his colleagues discuss the current state of knowledge in this area. Work over the last decade has led to the identification of the specific optimum and damaging temperatures of many important food crops. It has also become clear that high night-time temperatures often have significant impacts on grain yield and quality.

In rice, mechanisms to avoid heat damage have been discovered, although these remain elusive in other crops. Improving crop resilience to high temperatures could build on these insights from rice, using physiological, genetic or molecular approaches. However, targeting these efficiently will require identifying the sensitivity of each stage of the vulnerable reproductive phase, preferably on a scale of hours.

Rice has also provided an example of the great value of crop wild relatives as a source of novel traits, including resistance to high temperatures. The early morning flowering trait has been systematically identified and, with new molecular tools, incorporated into popular cultivars. Similar approaches could be used in wheat, and wild varieties are known to have considerable gene-associated variation in heat tolerance. New technologies have also increased the scope and ease of phenotyping (i.e., measurement of plant traits), and promising methods include the development of molecular biomarkers through 'omics' approaches such as lipidomics or metabolomics (i.e., measurement of lipids or metabolites). Another approach is the use of sensors to enable field-based high-throughput phenotyping, which is currently being tested by Professor Robert Aiken and his colleagues.

Management Strategies for Global Solutions

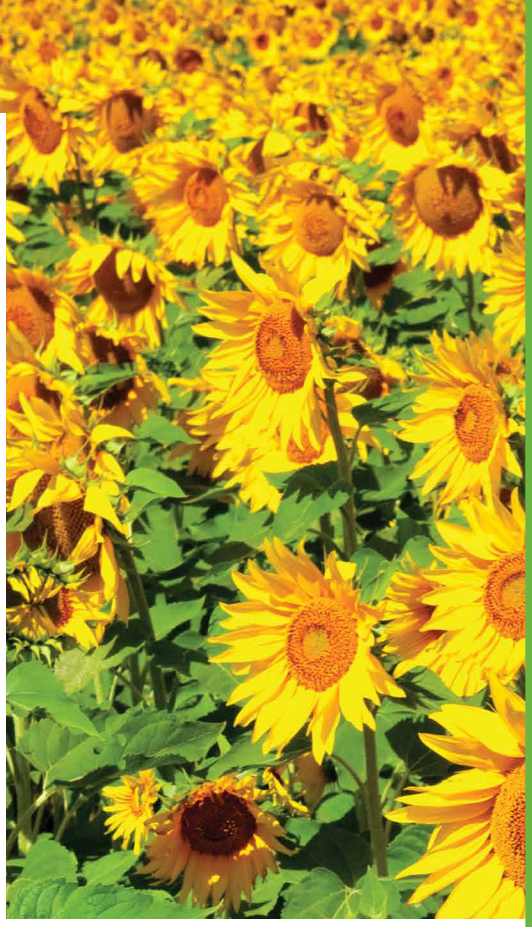
While these new methods promise exciting improvements in productivity, there is still considerable scope to boost yields through optimising traditional cultivation techniques, especially in developing countries. Professor Prasad is particularly interested in the application of innovative farming systems research to improving livelihoods of smallholder farmers. He recently became the Director of the Feed the Future Innovation Lab for Collaborative Research on Sustainable Intensification (SIIL) at KSU, which explores routes to increase agricultural productivity and income for smallholder farmers in Africa and Asia. Supported by USAID, the SIIL goals are reducing global hunger and poverty.

Together with research through multiple Innovation Labs in collaboration with the Savanna Agricultural Research Institute in Ghana, Professor Prasad and his team developed several improved agricultural practices to enhance productivity in West Africa. They assessed the impacts of conservation methods of cultivation on crop productivity and soil health – these methods include the use of crop rotations, crop residues, cover crops, minimum or no tillage, nutrient management and water harvesting. One of their recent projects focussed on maize and soybean cultivation in Ghana, and sorghum, millet, cowpea and peanut in Mali. The team showed that minimum tillage produced the same yields as conventional tillage, while saving on labour costs. Inorganic fertiliser and planting maize on tied ridges rather than in flat beds improved yield. Application of inorganic phosphorus fertiliser increased yield of soybean, cowpea and groundnuts.

Professor Prasad and his team are continuing their research on improving farming systems focused on yield improvement and efficient use of resources in multiple countries in Asia and Africa, and hope that their findings can be extended from the local experimental level to the wider regions, leading to larger-scale, sustained benefits in agricultural productivity.

CROP YIELD AND PROFITABILITY IN WATER-LIMITED CONDITIONS

Restricted water availability can determine yields and change the profitability of growing comparable crops. **Professor Alan Schlegel** compares the effects of different agricultural practices on yields in limited-irrigation and dryland crop systems.



Irrigation Levels Affect Relative Crop Profitability

One of the challenges facing agriculture as a result of increasing intensification and population density is the greater pressure on groundwater supplies. Climate change will also affect this, through increasing the unpredictability of rainfall and hence the recharge rates of groundwater. In the west-central areas of the Great Plains in the US, covering parts of Kansas, Nebraska and Colorado, the main source of irrigation water for agriculture is the Ogallala aquifer. This system is currently over-exploited and supplies declining volumes of water. Maintaining crop yields in this region will require improving the efficiency of water use in agriculture, and, with global freshwater supplies under pressure, insights gained from research here could be applied to other areas.

As the availability of water for irrigation decreases, farmers may consider applying lower levels of irrigation to their crops. Certain crops are less affected by water scarcity, and may become more appropriate to the new conditions. However, the decision to plant a particular crop is influenced by its profitability, which depends on its market value as well as its yield. Understanding how the profitability of common crops changes under different irrigation conditions is important to inform crop choice and higher-level planning to regulate water and food supplies.

Professor Alan Schlegel and his team at Kansas State University carried out a study to compare the yield and profitability of four common Great Plains crops under three different irrigation scenarios – of 127, 254 and 381 mm of irrigation water annually. These crops – corn, grain sorghum, soybean and sunflower – are all grown in the summer and compete for farmers' resources. The results of the study, published in 2016 in the *Agronomy Journal*, show that while corn is the most profitable crop at higher irrigation levels, its profitability advantage is lost at lower levels. However, its yield by grain weight per area exceeded that of sorghum, soybean and sunflower at all irrigation levels.

Local crop selling prices will determine the profitability under different irrigation scenarios, and this study was based on prices in Kansas. The team's work also sheds light on the circumstances under which there are economic grounds for increasing irrigation. Corn yields increased with all irrigation increments. For soybean, it was profitable to increase irrigation to 381 mm, but to a lesser extent than corn. For sorghum and sunflower, irrigation beyond 254 mm was not profitable. Although the estimates of profitability will vary depending on input costs and sale prices, the differences in irrigation profitability for corn and soybean, compared to sorghum and sunflower, are in line with the results of previous studies.

Limited Irrigation May Favour Continuous Corn Monocultures

Another piece in the puzzle of improving crop productivity in irrigation-limited conditions is the role of crop rotation. This is an ancient practice that may increase yields through a variety of mechanisms, including improving soil structure, reducing weed numbers, enriching soil nitrogen levels and others. Most research on crop rotations has focussed on maximising yield under rainfed conditions. There is less guidance available for farmers on which crop sequences are most suited to limited-irrigation conditions, such as those common in the central Great Plains.

Professor Schlegel and his colleagues performed an experiment to fill this gap, comparing the yields and profitability of corn

grown in three different crop rotations, or continuous corn. Published in the *Agronomy Journal* in 2016, their results demonstrate that while the continuous corn rotation had lower relative yields in comparison to the corn in the diverse rotations, its yield over the whole rotation cycle was greater. Using the local price for the four crops and the cost of production of the four cropping systems, continuous corn was the most profitable.

The relatively lower yield of the corn cycle in the continuous rotation was considered a consequence of the higher water use of corn compared to the other crops, which affected grain development. This shows that continuous monocultures may be the agricultural system preferred by farmers under limited irrigation scenarios, as profitability is often the main criteria in determining agricultural practices. However, the process of improving resilience to climate change should also consider environmental impacts and integrate their value into production systems in order to avoid decision-making based on short-term, economic gains.

The Role of Rotation Length in Wheat Yield

Planting crops in diverse rotations also has great potential to sustainably improve yields in dryland regions that rely on rainfall. In the Great Plains, the most common dryland wheat rotation used to be a two-year wheat-fallow system. However, replacing the fallow with another crop may lead to more efficient use of land and water inputs. Advances in available resources, such as improved herbicides, mean that it is now possible to plant wheat in rotation with warm-season crops such as sorghum or corn. The most popular rotation in the central Great Plains is now a three-year cycle with wheat, a warm season crop and fallow (WSF). Alternative rotations are also used, and longer rotations with two instead of one year of certain crops may offer benefits in terms of yield, profitability and lower required inputs.

Professor Schlegel and his colleagues conducted one of the first long-term studies of the yield of two four-year wheat rotation systems, as well as their water use and water productivity, compared to those of continuous wheat (WW) and of the popular three-year WSF rotation. The two mixed rotations were wheat-wheat-sorghum-fallow (WWSF), and wheat-sorghum-sorghum-fallow (WSSF), and the study was conducted from 1996 through 2015. Their results, published in 2017 in the *Agronomy Journal*, show that growing wheat in rotation with sorghum and fallow increased the overall grain yield relative to continuous wheat.

Wheat grown after fallow, and sorghum grown after wheat, led to higher grain yields, crop biomass, soil water and water productivity than wheat grown after wheat or sorghum grown after sorghum. However, the four-year rotations had similar profitability to those extrapolated for the three-year WSF rotation, so increasing the intensity of cropping does not automatically increase profitability. The relative water use by different crops in rotations affects the yield throughout the rotation cycle, and should be considered in planting decisions together with the yield potential and market value.

Meet the researchers

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Professor Robert Aiken received a PhD in Soil Biophysics at Michigan State University in 1992, as a USDA Water Science Fellow at the Department of Crop and Soil Sciences. He then worked as Post-Doctoral Soil Scientist in the USDA/ARS Great Plains Systems Research Unit in Colorado, before moving to the USDA/ARS Central Great Plains Research Station in 1995. Since 1998, he has worked as Research Crop Scientist at the Kansas State University Northwest Research-Extension Center, and is also Associate Professor. He also advises the Central High Plains Working Group, the North Central Regional Committee 1200 Regulation of Photosynthetic Processes and the Sunflower Crop Germplasm Committee.

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Professor P. V. Vara Prasad obtained his PhD in Crop Physiology from the University of Reading in 1999. From 2000 to 2005 he worked as Postdoctoral Research Associate in the Agronomy department of the University of Florida. He then moved to Kansas State University to take up the role of Assistant Professor in Crop Ecophysiology, becoming Associate Professor and Director of the Centre for Sorghum Improvement in 2009. In 2013 he became Professor of Crop Ecophysiology, and in 2014 he also became Director of the USAID Feed the Future Innovation Lab for Collaborative Research on Sustainable Intensification, both at Kansas State University. In 2016, he became University Distinguished Professor a lifetime title that represents the highest honor KSU can bestow on its faculty.

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KANSAS STATE UNIVERSITY

ADAPTING DRYLAND CEREAL PRODUCTION TO CLIMATE CHANGE

Cereal production is vulnerable to climate change. **Professor Sanford Eigenbrode** is the Project Director of Regional Approaches to Climate Change for Pacific Northwest Agriculture (REACCH), a project that was initiated to address the challenges facing cereal systems in the Pacific Northwest USA. The project team organised a conference in November 2015 to share their findings and consolidate knowledge from experts on five continents. Here, we present the findings from this conference, which offer a range of solutions and challenges for adapting cereal systems worldwide to climate change.

The REACCH Project

Climate change presents a serious threat to global food security. Major staple crops systems face challenges worldwide due to ongoing and projected changes in precipitation and temperature. Among the most vulnerable are cereal systems in semi-arid regions, which are extremely vulnerable to fluctuations in precipitation and elevated temperatures. In the major semi-arid cereal growing area of the inland Pacific Northwest (Idaho, Oregon and Washington in the USA), scientists predict accelerating rising temperatures, accompanied by drier summers and wetter winters.

The challenge is to retain productivity in this rain-fed cereal system through crop resilience under changing water and temperature regimes. This can involve diverse solutions, such as reducing soil erosion and restoring soil fertility through different production practices and crop rotations, exploiting genetic resources to improve heat tolerance, water use efficiency and nitrogen use efficiency of cereals, and maintain or improve resistance to pests and diseases.

Addressing the challenge of climate change therefore requires a multidisciplinary approach that integrates knowledge from across many scientific domains. To achieve this, the United States Department of Agriculture's National Institute of Food and Agriculture (USDA-NIFA) funded a coordinated agricultural project called 'Regional Approaches to Climate Change – Pacific Northwest Agriculture' (REACCH – https://www.reacchpna.org). This seven-year project was led by a team of researchers from the University of Idaho, Washington State University, Oregon State University, and the USDA's Agricultural Research Service.

'The REACCH project was initiated in 2011 to ensure sustainable cereal production in the inland Pacific Northwest,' explains the Project Director, Professor Sanford Eigenbrode. 'Participants from many disciplines related to agricultural, climate, socioeconomics, and information sciences engaged in an integrated research, education, outreach and extension effort to study complex cereal production systems and their responses to drivers of change.'

Adapting Cereals to Climate Change

To share their findings with specialists working on cereal systems globally, the REACCH team organised a conference, entitled 'Transitioning Cereal Systems to Adapt to Climate Change' (TCSACC – https://aridcereals.nkn.uidaho.edu), at the Minneapolis Convention Centre in November 2015. Experts from 17 countries attended the conference, with backgrounds ranging from agronomy, plant physiology, crop protection, plant breeding and genetics, to computer modelling, sociology and economics. The conference emphasised the integration of diverse aspects of complex agricultural systems.

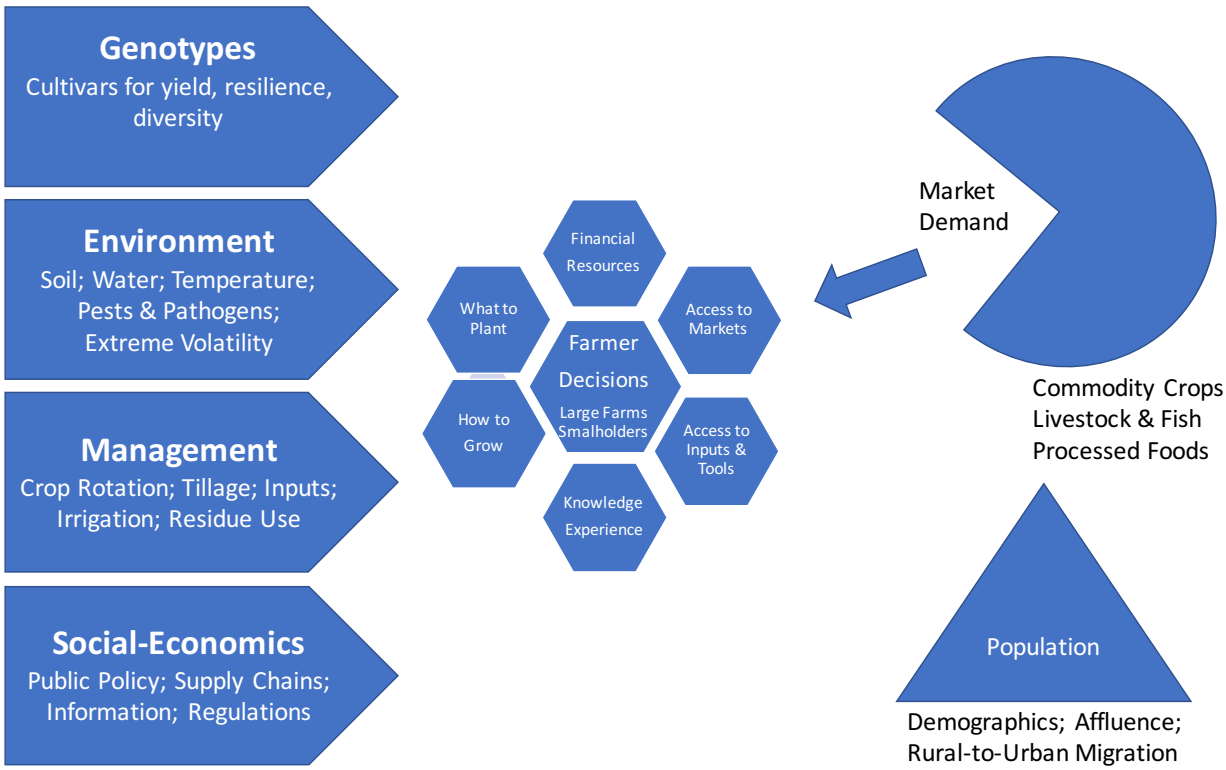
The goals of the workshop-style conference were to strengthen the global network of researchers addressing the impacts of climate change on semi-arid cereal-based systems, to share approaches to achieve multidisciplinary collaboration towards advancing climate change resilience in cereal systems, and to identify the necessary elements of a collaborative research agenda for advancing global food security.

In a paper published this year in *Frontiers in Ecology and Evolution*, Professor Eigenbrode and his colleagues Professor Patrick Binns and Dr David Huggins summarised the conference themes and conclusions, and suggested steps for action.

Water Scarcity

Cereal production systems in semi-arid habitats are mainly limited by available water. Climate change predictions suggest that water will become scarcer in many regions where cereals are grown. Agronomic approaches to increase water use efficiency include fallow periods, prudent tillage and residue management, and novel crop rotations. The REACCH project, for example, experimented with using different rotational crops (such as winter canola, winter pea and triticale), and water conserving technologies such as harvesting equipment that leaves enough post-harvest biomass in the field to trap winter moisture.

Professor Eigenbrode and the team noted that more research is needed to identify



Schematic representation of cereal production as a social-ecological system. The domains of environmental conditions of soil quality, precipitation, fresh water access, temperatures, pest pressures and extreme weather events, genotype properties of cultivars, annuals, perennials, root structure; biological N fixing stress tolerance, agronomic management of crop diversity, tillage, input use, animal integration residue retention, and social-economic factors that affect market demand, price signals, capital investment, public incentives, regulations, research; and cultural customs and attitudes all interact to establish the context, motivation and resources that influence farmers' decisions concerning how to adapt to changing climate regimes.

and evaluate the ability of alternative rotational crops and agronomic practices to improve the efficiency of water use. Climate projections can help farmers make decisions about which rotational crops to plant, while the availability of a wider range of economically-viable crops and methods would facilitate this type of adaptive management.

Plant breeding research is enabling cereals to improve their water and nutrient use efficiency, and drought resistance. However, TCSACC participants stressed the need for further improvements in the global coordination of these efforts. Studies have shown that when an approach that combines genetics (G), environment (E) and management (M) is deployed, yield and quality increases are greater than for approaches based only on a single technological innovation. At TCSACC, it was agreed that this approach should be extended to include societal factors that influence management decisions (i.e. G × E × M × S).

Discussions at the TCSACC conference also identified key needs for advancing crop adaptation to climate change, including improved technology for genomic selection, universal data-sharing, and better utilisation of cropping system models.

Managing Data

Professor Eigenbrode and his colleagues believe that developing climate-resilient cereal varieties will require the integration of empirical and modelling approaches. This is because it is impractical to conduct sufficient empirical studies replicated across variable production landscapes. Future solutions will therefore combine virtual cropping system models with field data collected using more accurate and affordable sensors and data acquisition systems. For example, the REACCH team used the CropSyst crop modelling system to guide their research on climate change and cereal production, which can be extended to evaluate the various rotational cropping systems needed for adapting to climate change.

In partnership with the University of Idaho's Northwest Knowledge Network (NKN), the REACCH team developed their own capabilities to acquire, store and manipulate data for semi-arid cereal production systems in the Pacific Northwest region. To optimise crop modelling, however, scientists need to draw upon the results of research conducted globally, through improved access to data.

Discussions at the TCSACC conference concluded that a key research restraint worldwide is the inadequate capacity and capability of existing data repositories to support accessibility to diverse data sets. The consensus was that new approaches are needed to acquire and manage diverse types of data relevant to entire crop production systems, and to share these across semi-arid regions. The data management system developed by the REACCH team was conceived as an eventual node within a broader collaboration to enable this type of data sharing.

Protecting Crops

Models for cereal production systems under pressure from climate change have often ignored associated changes in pressure from

insect pests, diseases and weeds. Attempts to do so are difficult, due to the complexity of these interactions. However, studies have shown that climate-induced stress makes plants more susceptible to pests, pathogens and competition from weeds.

In their review focusing on the insect pests of wheat and climate change, Professor Eigenbrode and his colleague, CSIRO scientist Dr Sarina Macfadyen found that only 12 species had been studied, mostly using specific approaches such as population modelling, although better understanding requires multiple approaches in combination. Similarly, more research is needed into the effects of climate change on pathogens and weeds in cereal systems.

Within the REACCH project, pests, diseases and weeds were framed within a broader systems approach. The team's ongoing work involves incorporating insect feeding into process-based crop models for wheat such as CropSyst – for example, the effects of projected climate change on the damage to yield caused by the cereal leaf beetle.

As identified at the TCSACC conference, researchers need to start filling certain knowledge gaps concerning pests and climate change in cereal systems, including coupling long-term records of pest abundance with historical climate records. Furthermore, agronomic practices such as alternative tillage, rotation and nutrient management schemes are also likely to influence pests, disease and weed risks, warranting their monitoring as part of evaluations of alternative practices designed to adapt to climate change.

Mitigating Greenhouse Gases

Climate change impacts crop productivity, but crop production also contributes to climate change. Therefore, alongside adapting cereal systems to changing climates, it is also important to minimise the negative impacts of agriculture on the climate system. Agriculture is responsible for around 11% of total greenhouse gas emissions worldwide, with approximately 65% of this due to nitrous oxide emissions from agricultural soils, exacerbated by poor agricultural practices. Effective adaptation practices could therefore achieve 'win-win' benefits in terms of crop yields and more efficient use of nitrogen fertilisers, resulting in both improved productivity and reduced nitrous oxide emissions.

Researchers at the TCSACC conference noted that improved monitoring of greenhouse gas emissions from different cereal production and nutrient management practices is needed to identify how emissions could be minimised. In the INPW, the REACCH team looked at conventional and alternative wheat production systems under different precipitation regimes, in chambers that enabled nitrous oxide emissions to be measured. Their models indicated that nitrous oxide emissions in the region are less than those estimated by the Intergovernmental Panel on Climate Change (IPCC), though recent data from other regions of the USA showed higher-than-predicted emissions.

Social and Economic Factors

Agricultural systems are also social-ecological systems. Successfully adapting to climate change will depend upon technological capabilities, the economic costs and returns of adopting new practices, and factors that govern the behaviour of producers. In northern temperate regions, farmers have often been reluctant to accept the validity of climate change, reducing their likelihood of adopting new

practices. Studies have found that perception of risk varies, and is influenced by the level of indebtedness, awareness of alternative practices, age and other factors.

Social and economic dimensions were incorporated into the REACCH project at several levels. For example, modelling crop performance under different climate scenarios enabled the research team to project impacts on economic returns. The team also carried out surveys that assessed farmers' attitudes and perceptions about climate change, to guide research priorities and communication strategies. A key take-home message from the TCSACC conference was that integrated agricultural research responses to climate change should strive to understand sociological forces and incorporate them into interdisciplinary assessments and strategies.

Going Forward

In the final section of their *Frontiers in Ecology and Evolution* paper detailing the outcomes of the TCSACC conference, Professor Eigenbrode and his colleagues concluded: 'Based on discussions within TCSACC and this review, progress to achieving more integrated and effective approaches for addressing the challenges of climate change in semi-arid systems will be accelerated by improved interdisciplinary and intersectoral integration that can address production at a comprehensive systems level.'

TCSACC participants endorsed the importance of nurturing existing networks of scientists working to help cereal systems in semi-arid regions transition to respond to the changing climate. Professor Eigenbrode has said that the collaborations established during the REACCH project will continue. Action Steps undertaken since the conference include the establishment of an international Expert Working Group on Wheat Agronomy and a 'Community of Interest' within the Agronomy Society of America that is focusing on adaptation strategies for dryland cereal systems.

In summary, the conference addressed and endorsed a list of eight needed components of actionable research going forward:

- Establish coordinated, large scale, transdisciplinary efforts
- Consider genotype x environment x management x societal interactions
- Improve integration among knowledge communities
- Consider global context of production systems
- Develop more inclusive cropping system models
- Enable comprehensive data management and data sharing
- Include landscape and ecosystem services perspectives
- Establish and support existing global networks

Meet the researcher

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Professor Eigenbrode is a University Distinguished Professor and Professor of Entomology at the University of Idaho. He received his education at Cornell University, where he gained his BS and MSc, and then his PhD in 1990. Following this, he worked as a postdoctoral scientist at Cornell University, the University of California and the University of Arizona. He became Assistant Professor of Entomology at the University of Idaho in 1995, and he has been Professor of Entomology in the Department of Plant, Soil and Entomological Sciences there since 2006. More recently, he became Adjunct Professor of Entomology at Washington State University. Professor Eigenbrode's main research interests are ecology, chemical ecology, biological control, insect-plant interactions, host-plant resistance, and virus-plant-vector interactions.

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COWPEA – AN ANCIENT CROP FOR MODERN CHALLENGES

Cowpea has been cultivated as a crop for thousands of years, and is well-placed to improve the sustainability of modern agricultural systems. As part of a NIFA-funded project, **Dr Louis Jackai, Dr Beatrice Dingha** and **Dr Mulumebet Worku** and their students at North Carolina A&T State University are carrying out research that could open up wider avenues to the cultivation of this uniquely versatile crop.

Cowpea as a Trap Crop & Pollinator Community Enhancer

Cowpea is a widely grown legume used both as a food crop for humans and animals, and as a cover crop to improve soil quality and suppress weeds. It is thought to have been one of the first plants to be cultivated by humans, and its seeds are a good source of protein and vitamins, playing an important role in traditional African, Asian and southern US cuisine.

Despite cowpea's long history in agriculture, scientists are still discovering surprising new uses for it. Dr Louis Jackai and Dr Beatrice Dingha are entomologists at North Carolina A&T State University. They are particularly interested in cowpea's wide range of uses, which include its potential to reduce pesticide application in agricultural systems through its use as a trap for insect pests.

Insect pests cause considerable damage to crops, and can reduce yields by over 90%. Currently, pesticides are widely used to control pests. With increasing recognition of the negative effects that can result from pesticide use, both on human health and on the environment, there is growing appetite for alternative pest management solutions. It has long been known that increasing the number of crop species grown on an agricultural landscape can suppress pest levels. One such strategy involves growing crops together in time and space, as intercrops or companion crops, with one crop that is highly preferred as the trap crop. This strategy diverts pests from the main (protected) crop to the trap crop, thus resulting in reduced damage to the main crop.

Dr Jackai's team has shown that the brown marmorated stink bug – an invasive species that has recently caused considerable damage to US crop yields – is particularly attracted to cowpea. This could make cowpea a suitable trap crop to grow alongside crops such as soybean. However, the interaction between crops can be complex, and may result in either an increase or a decrease in the pest load depending on the insect-plant interrelationships. Dr Dingha is currently leading the team's research to improve our understanding of how cowpea might perform with different main crops, such as corn, peppers, soybean and others. Dr Jackai and his students have also shown that some cowpeas can also be used as an intra-specific trap crop used to protect other cowpea varieties from the brown marmorated stink bug.

Cowpea also offers another benefit when grown with different main crops, especially those that are cross-pollinated. Although cowpea flowers do not need insects for pollination, they produce large amounts of nectar, attracting and supporting pollinators such as bees and butterflies. This could increase the yield of insect-pollinated crops such as squash, melons and related crops, as well as improving ecosystem health through boosting pollinator activity. Dr Jackai and his team think cowpea might even make a welcome aesthetic splash in the growing trend of urban agriculture, with its edible fruit (fresh cowpea pods) preceded by brightly coloured flowers that appear synchronously and beautify our landscapes. 'We have identified two varieties we think would be particularly useful for urban spaces, with their low growth habit and beautiful blooms that brighten our surroundings, yet which can also be used for human food,' Dr Jackai says.

Damage to cowpea pods caused by the Brown Marmorated Stink Bug

The Brown Marmorated Stink Bug

Better Yields, Storability and Profits with Vegetable Oil

Cowpea's attractiveness to insects is a double-edged sword, and has historically resulted in low yields when grown as the main crop or monocrop. The entire plant, including foliage, pods and seeds, is susceptible to attacks by a range of insect pests, including the leaf-footed bug, cowpea curculio and several types of stinkbugs. Dr Jackai, Dr Dingha and their colleagues have observed that although there are some areas with low levels of pest infestations, many cowpea farmers use large amounts of insecticides to control insect damage, sometimes spraying up to three times a week in locations with multiple pests and/or high infestations. This practice is damaging to human health and the environment, affecting the populations of beneficial insects and other animals that are important for ecosystem function, such as the pollinators mentioned earlier.

Dr Jackai and Dr Dingha's work on integrated pest management solutions for cowpea crops in the US includes exploring other alternative methods of pest control. They have shown that carefully choosing the planting date can reduce the populations of cowpea pests, avoiding the need for insecticide use. In the case of the brown marmorated stink bug and related pests, this effect is more pronounced with early planting once environmental conditions become favourable, since pest populations tend to increase as the season progresses.

Another promising approach to reduce insect infestation in stored cowpea stands out for its simplicity and cost-effectiveness: treating stored cowpea seeds with vegetable oil. The research team has been exploring this approach – assessing how well different types and amounts of commonly available vegetable oil such as peanut, soybean, canola, truffle, and others can control populations of a particular beetle that can reduce stored cowpea to dust within a period of three months. The team found that treating cowpeas with oil can greatly reduce the beetle's ability to deposit eggs on them, leading to a decrease of over 95% in the number of adults emerging from the seeds.

The team also observed a strong increase in adult beetle mortality. Even small doses of oil were found to be effective in certain cases, and there was little impact on the percentage of seeds that germinated. The team is now planning a range of further studies to identify the optimal procedures for oil treatments in small and medium-size farm enterprises. This research builds on, and strives to standardise, earlier work by several researchers in the field.

They hope that their findings will greatly improve the yield and profitability of cowpea, especially for small-scale farmers in their state of North Carolina, as well as in other cowpea producing states in the US and beyond.

Surprising Health Benefits

Cowpeas are rich in a range of compounds, which could affect a variety of biochemical pathways. Dr Jackai recently collaborated with Dr Mulumebet Worku, an animal scientist and molecular biologist and her former graduate student, Dr Sarah Adjei-Fremah, both also based at North Carolina A&T State University, to explore the effects of feeding cowpeas to livestock. The researchers were able to show that phenolic extract from cowpea has beneficial effects on processes associated with immunity, homeostasis and adipogenesis (transformation of fat cells) in cows. Their results were published in 2016 in the *Journal of Applied Animal Research and Journal of Agricultural Science.*

Drs Worku, Jackai and Adjei-Fremah also collaborated on another study investigating the effects of cowpea on livestock production and welfare. Published in 2016 in the Journal of Animal Science, their findings indicated that compared to a control diet of pearl millet, feeding cowpea forage to goats appeared to improve their resistance to internal parasites, leading to increased body weight and a lower level of parasite eggs in their faeces. Interestingly, the goats' intestinal microbiome was also altered, and contained a reduced methanogen population. As methanogen bacteria are responsible for producing the greenhouse gas methane, feeding cowpea to animals could help to reduce the carbon footprint associated with livestock farming.

The team noticed that goats' immune response biomarkers such as PGE2 and cytokines were modulated, meaning that cowpea's various beneficial effects possibly act through boosting the animals' immune responses. The team's studies could open up previously unexploited routes to improving livestock health and optimising meat production.

However, the biochemical effects of cowpea cultivars can vary, depending on their molecular composition. Some cultivars contain certain phenolic compounds which can be bitter, toxic and interfere with digestion, making them less appropriate for consumption. Other compounds may be present that have beneficial effects, such

Eggs of the cowpea storage beetle are laid on seeds of shelled peas. When seeds are treated with vegetable oil many eggs may not develop into adult beetles, thus protecting cowpeas from damage from a future generations of beetles. Uniform and effective oil application protocols are being developed to ensure all seeds are covered with a thin film of oil.

as isoflavones. Isoflavones are naturally occurring compounds that are associated with protection against a range of human diseases.

In another study, Drs Adjei-Fremah and Worku explored this and found that cowpea extract reduced the proliferation of triple-negative breast cancer, a very severe form of the disease common among people of African descent. It is thus important to identify the specific characteristics of cowpea cultivars, with a view to using them for pharmaceutical treatments.

These studies have contributed to defining the molecular composition of cowpea cultivars. Future plans include carrying out experiments to further understand the effects different cultivar compositions have on physiology. For humans, this could open up routes to new medical treatments. For agriculture, the researchers' work will lead to improved production and use of cowpea, and enhanced livestock farming. In turn, this greater efficiency could allow the conservation of the Earth's limited environmental resources.

Towards Better Livelihoods

Cowpea has a number of other traits that make it especially suitable for low-income farmers in challenging environments. Thanks to its ability to convert atmospheric nitrogen to useful nitrogen compounds (nitrogen fixation), cowpea does not need nitrogen fertilisers and can thrive on lower quality soil. This also makes it a good crop to grow as a cover crop or during crop rotation, as it enhances soil fertility and organic matter, while preventing erosion and suppressing weed growth.

Cowpea can tolerate a range of conditions, including high temperatures and drought. This property is increasingly attractive in the face of climate change, which is extremely likely to bring greater unpredictability in weather patterns. With its hardiness and versatility, cowpea is an excellent crop for integrated farming systems that incorporate both crops and livestock. This could offer a route towards greater sustainability, and improve the yields and profitability of small farms.

Although world cowpea production has increased, production in the US has declined dramatically. Drs Jackai, Dingha and Worku believe that there is great potential for further expansion of cowpea cultivation, perhaps to levels more than double the current levels. The crop could be particularly productive in states such as North Carolina, which has recently seen damaging droughts that are expected to increase in frequency with climate change.

The state also has a large population of displaced tobacco farmers, who might find cowpea an appropriate alternative crop.

Drs Dingha, Jackai and their research team are exploring ways to reduce the barriers to cowpea cultivation in North Carolina. They believe that modern technological advances and new approaches to pest management could improve yields, as could a better understanding of the great diversity in cowpea cultivars and their unique advantages. Their work focuses on assessing cowpea cultivars for their cultivation potential, based on insect resistance, their attractiveness as a trap crop to attract insect pests and support pollinators and their ecological services, ability to enrich soil nitrogen levels, and to provide ground cover. The 492-acre research farm at North Carolina A&T State University provides an ideal space for their experiments, and their preliminary findings have allowed them to identify several cultivars which appear to show promise for the next generation of cowpea crops.

Drs Jackai and Dingha also emphasise the importance of making cowpea cultivation affordable to small-scale farmers with limited incomes. Subsistence farming is an important way of life in developing regions of the world. In the US, small farms still provide valuable income to their owners – 90% of the farms in North Carolina are less than 50 acres in size. While these farmers cannot compete with larger farms in the cultivation of most crops, fresh pea cultivation favours small-scale and local production, and peas can be sold through avenues such as farmers' markets. As such, cowpea seems to have great potential for small farms.

Overall, cowpea can improve farm productivity and profitability through its potential to boost pollination of the main crop, enrich soil fertility and act as a trap crop, while helping to reduce environmental impacts associated with intensive pesticide use. Dr Jackai and Dr Dingha are working directly with local farmers to test the economic feasibility of incorporating cowpeas into small farming systems. They believe that the multi-talented cowpea could play a role in improving livelihoods. This would contribute to addressing the priorities of the National Institute of Food and Agriculture (NIFA) of the USDA (who sponsored most of their research), through sustainably increasing global food security. Such research offers essential insights in the ongoing battles against world hunger in the face of an increasing human population and climate change.

Meet the researchers

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Dr Beatrice Dingha obtained her PhD in Entomology from Auburn University in 2005. From 2006 to 2010, she worked as Postdoctoral Research Associate at Tuskegee University, before moving to NC A&T State University in 2010 to take up the role of Research Associate in the Family and Consumer Sciences Department. In 2015, she joined the Natural Resources and Environmental Design Department as a Research Scientist conducting research on urban and agricultural pests. Her research focuses on food safety and food security with long-term goal of increasing agricultural sustainability using IPM.

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Dr Louis Jackai received his PhD in Entomology from the University of Illinois in 1978. He then worked as Postdoctoral Fellow at the International Institute of Tropical Agriculture in Nigeria, becoming Principal Scientist in 1980. From 2000 to 2008 he was Professor of Entomology at Tuskegee University. He joined NC A&T State University as Chair and Professor of Entomology in 2009 and in 2015 became Graduate Coordinator of the Department of Natural Resources and Environmental Design. Dr Jackai conducts entomological research involving the use of insect nutritional ecology in the development of safer and more sustainable approaches for field and postharvest insect pest management. His ongoing research strives to increase environmental safety, food security and agricultural sustainability through a focus on pest management dynamics in vegetable production systems in small rural and urban landscapes.

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Professor Mulumebet Worku received her PhD in Animal Sciences from the University of Maryland in 1993, and started working at the National Institutes of Health as Staff Fellow, moving to the USDA Agricultural Research Service in 1996, where she took up the role of Research Biologist. In 1998, she joined the faculty in the Animal Science Department at NC A&T State University, where she worked from 2003 as assistant, associate and, since 2009, full professor. Her research has contributed to the definition of the role of genetic diversity in inflammation with current research interests focusing on studies in genomic diversity and innate immunity for the development of novel biotechnologies for global food security.

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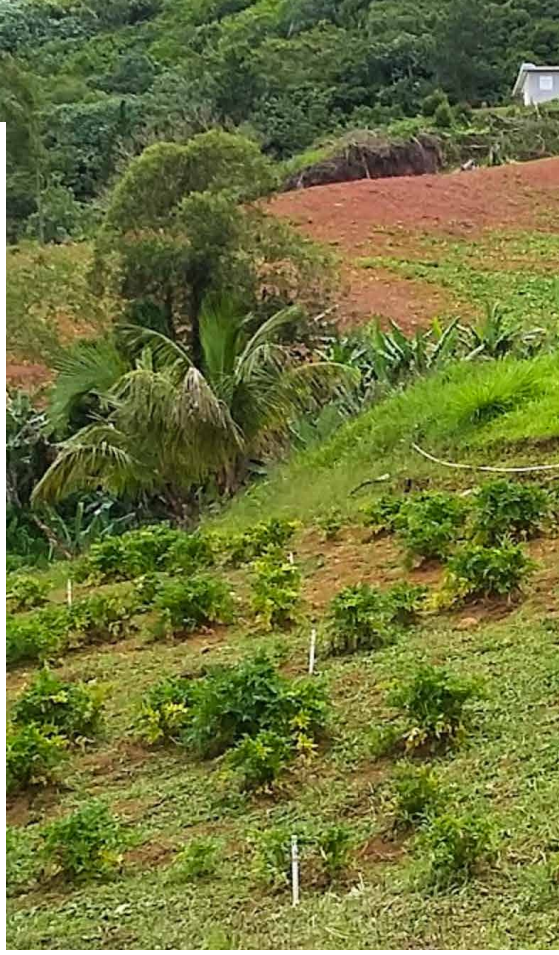
NORTH CAROLINA AGRICULTURAL AND TECHNICAL STATE UNIVERSITY

SOLVING THE PROBLEM OF APIO *(ARRACACIA XANTHORRHIZA)* CORM ROT DISEASE



Essentially un-researched until now, corm rot disease has had detrimental effects on the Puerto Rican agricultural industry. Here, **Dr Martha Giraldo** and her research team at the University of Puerto Rico collaborate with local farmers to investigate the source of this persistent pest.

Arracacha, or *Arracacia xanthorrhiza* Bancroft, is one of the most ancient, tuberous crop plants of South America. Resembling a slightly plump parsnip, it presents celery-like shoots, growing up to two meters in height. This similarity landed the plant with its nickname 'apio' in Puerto Rico – that's Spanish for celery by the way. Its pleasant flavour, low maintenance, easy digestibility, superior starch and high levels of P-carotene, ascorbic acid, Vitamin A, phosphorous and calcium, put this perennial plant in high demand.

The main body of apio comprises a large 'corm', accompanied by a multitude of much smaller, immature cormels. These are broken up for use as new vegetative seeds or propagation material during crop practices in the agricultural industry. The corm sits at the earth-air interface, with its collection of edible roots plunging downwards, and the celery-esque shoot rising sky-high.

The local people in the mountains of Puerto Rico have been cultivating apio since 1903, giving it traditional status throughout the region. Up until 2004, the farming community prospered, with a building demand for apio both internally and externally. This generated relative wealth and affluence, especially in the central region where humidity and highland terrain make suitable crop plants difficult to come by.

Since 2004, however, apio farmers have witnessed mammoth revenue decline, perpetrated by a 'corm rot disease'. This under-explored ailment causes rotting to both the corm and roots, alongside wilting of the shoots. With most farmers in the central region experiencing resultant 50–100% yield losses, corm rot has caused the largest negative impact on apio farming over the past decade. Each year, post-harvest decisions become more difficult to make: how much to sell? how much to retain for propagation? One small mistake results in a financially formidable year ahead.

Farmers who continue to cultivate apio run the risk of corm rot-override. Even with its price and import continuing to rise, year on year, apio has become a challenge for Puerto Rican farmers. With a germination rate of less than 45% in the region, the area of farmland in central Puerto Rico decreased from 196.5 to 82.5 hectares between the years of 2002 and 2012.

Anguish and Uncertainty

To say that research into the cause of corm rot disease is lacking is an understatement. With only four published papers on the topic, dating all the way back to 1999, Dr Martha Giraldo, of the University of Puerto Rico, was determined to implement strict research into potential corm rot-causing pathogens, as an attempt to bring structure to this agricultural immediacy.

Multiple conflicting conclusions as to the disease-causing agent have so far been put forward: some claim a combination of the oomycete, with fungus-like *Phytophthora palmivora, Rhizoctonia* and *Fusarium* causes the disease, while others are confident that *Erwinia* bacteria are acting alone. More progressive studies show that two types of nematode, *Meloidogyne* and *Pratylenchus*, are potential instigators. Optimal

preventative measures differ significantly for each proposed conclusion, so how can we take action with so much uncertainty?

Here, we detail how Dr Giraldo's research team strive to provide corm rot-free tissue culture and identify a nematode species that is prolific in affected apio fields. As well as providing farmers with propagation material, tissue culture allows the genetic diversity in apio plants to be conserved. Pathogenicity studies performed by the team allow them to offer better advice concerning agricultural practices, potentially re-revolutionising the use of apio as a sustainable crop plant in central Puerto Rico.

Characterising the Cause

From four apio farms of two Puerto Rican mountain municipalities, Barranquitas and

Orocovis, Dr Giraldo and her team collected an array of samples: symptomatic apio tissue, asymptomatic apio tissue and soil. They assessed the presence of nematodes, bacteria and fungi, especially the plant-parasitic varieties.

Individual nematode populations were extracted from samples using Baermann's funnel – a scientist's fancy version of a filter. The team then extracted living nematodes from the soil, and examined and identified them under a microscope. By measuring discreet portions of soil at a time, and considering the degree of corm rot-presence where each sample was taken from, Dr Giraldo's team were able to get an idea of how pathogenic each species was.

On one of the farms, the team collected a more extensive selection of soil samples. Not only did the researchers test these for nematodes, but also for plant pathogens, bacteria, oomycetes and fungi. These microbial masses were grown up to form colonies in the laboratory, and Dr Giraldo's team of scientists determined the density of each and its relation to corm rot.

The team identified nematode populations on all four farms: *Rotylenchulus reniformis* (85.6%), *Helicotylenchus multicinctus* (8.2%) and *Pratylenchus coffeae* (0.7%). *Rotylenchulus reniformis* was particularly present on Luis Rivera Berrios' farm in Barranquitas, and Luz Torres' farm in Orocovis. These two farms also experienced the most devastating yield losses, implicating *R. reniformis* as a causal agent for corm rot disease.

A Tally of Treatments

Upon identifying *R. reniformis* as a potential corm rot-causing agent, Dr Giraldo and her colleagues implemented an integrated management experiment throughout Luis Rivera Berrios' Barranquitas fields. They did so by applying eight different treatments in specific spaces where corm rot had prevailed in the past.

The first involved solarisation prior to apio-introduction: mulching the target soil, before topping it with a transparent heat trap layer in the form of polyethylene. This provided a cost-effective method of 'bleaching' the treated soil, exclusively utilising energy from the sun. The second apio-enhancing strategy involved the use of biological and chemical products as agricultural microbicides, applied directly to the topsoil used for planting. Third, the team took the solarisation protocol to the next level, transferring this compost into the growing region, before any growth had started.

Treatment number four was applied to the propagation material itself. Vydate-L, which kills insects and nematodes, was applied 24 hours before planting. This allowed time for the chemical to establish itself, for more successful sprouting. Tagetes, a pest and pathogen antagonist, and green manure, made up the fifth treatment. Upon

application, the team allowed a two-month period before starting the apio propagation process. In the sixth treatment group, the team used sweet corn as a nematode tolerant and a short cycle crop to harvest between each apio yield. This technique is similar to crop rotation – with corm rot-specific pathogens being put off by periodic sweet corn presence.

The seventh treatment involved an integrated control management system, comprising a combination of the second, third, fourth and fifth treatments mentioned above. The team's eighth group provided a control for comparison – farming in the traditional style, which simply comprised of crop fertilisation and regular weeding. This control group was vital for the researchers to determine the effectiveness of each implemented action.

The team worked out the effectiveness of each of their seven treatments in comparison to that observed in the eighth control group. They used statistical tools to analyse all relevant factors: pathogen populations present, disease frequency, average corm weight, and total mass of harvestable material. The team tested each group before each treatment was applied, on the day of planting, mid-crop cycle, and during harvest. Dr Giraldo could then paint a picture of the fluctuations in pathogenic presence for each investigated method, gaining an idea of which treatments to enforce.

Propagation Protocol

Tissue culture involves growth of new plant matter from tissue samples taken directly from the field. For success, four distinct steps are required: establishment (specific tissues are isolated from a disease-free mature plant); multiplication (growth on nutrient-rich media); pre-transplant (stimulation of root growth in immature plantlets); and hardening (preparation of the plant for true growth conditions). Up until this point, the plant will never have experienced soil, sunlight or weather, and the final hardening phase often provides the most complex of all the challenges.

Research regarding the development of *full* protocols for disease-free apio growth is lacking. Working at the Agricultural Experimental Station in Corozal, Dr Giraldo was determined to establish a complete protocol, denoting best practices in apio tissue culture growth. She intended to supply specific method variations to support differences observed between local varieties, as a means of generating more readily available corm rot-free propagation material.

The main challenge presented itself to Dr Giraldo and her colleagues in the form of behavioural variation between local apio varieties. Further factors that inhibited their progress included the successful development of plantlets, and simulating root development before transplanting the plantlets.

Using pathogenicity tests for validation, the researchers were able to establish a useable protocol for producing, multiplying and successfully growing an adequate volume of apio. They did so using ethanol, Clorox (a household bleach) and Tween (an emulsifier) to sterilise the tissue, followed by multiplication in a general culture medium called 'Murashige and Skoog'.

Once established, this method allowed the team to consistently isolate non-contaminated tissue, allow it to multiply at a rate of 3.5–4 new shoots over each three-week period, and produce 95% rooting plantlets, in the lab. Dr Giraldo's ultimate goal is to provide disease-free arracacha propagation material eventually derived from these plantlets to be used for consistent successful growth on Puerto Rican farms.

Education and Outlook

Upon extensive research, experimentation and investigation, Dr Giraldo and her team have landed upon a series of solutions to their primary concerns. They are developing workshops for agricultural extension agents and local farmers as a means of communicating the integrated crop management systems required for effective disease control. These workshops will also provide detailed information regarding corm rot disease as a whole, allowing farmers to make more informed apio-based decisions when cultivating their land. Training meetings and agricultural field days have been organised for local farmers, targeted at promoting continued use of apio as a crop.

Alongside these positive developments, several unanswered questions remain yet to be answered. Dr Giraldo refuses to cease her studies until every hurdle has been hopped and all barriers broken.

Existing shortfalls lie in the realms of devising consistent successful tissue culture procedures. Two of the team's main focuses include developing specific tailored methodologies to account for the variation in local apio varieties, and stimulating root growth before transplanting plantlets. The team is also working to provide a method for reliable hardening.

Questions uncovered throughout the project have led Dr Giraldo to embark on additional enquiries alongside her ongoing studies. A new aim involves the construction of a decontamination protocol for farmers whose propagation material is already infected. This could provide a means of role reversal as the Puerto Ricans put a stop to corm rot.

Meet the researcher

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Since graduating with a degree in Biology from the Universidad del Valle, in 1996, Dr Martha Giraldo has displayed a consistent passion for developing solutions relating to agricultural plant pathology. Her career since then has included a Research Assistant role at the International Center for Tropical Agriculture, a PhD in Plant Pathology, and her current position of Assistant Professor in the Department of Agro-Environmental Sciences at the University of Puerto Rico. In receipt of multiple internationally recognised certifications, such as the American Society of Microbiology's 'Outstanding Young Investigator Award', Dr Giraldo has certainly brought value to the scientific community. Having acted as reviewer for five relevant scientific journals, been invited to speak at numerous conferences and seminars, and produced an extensive list of published papers, Dr Giraldo's contribution to agricultural science is second to none.

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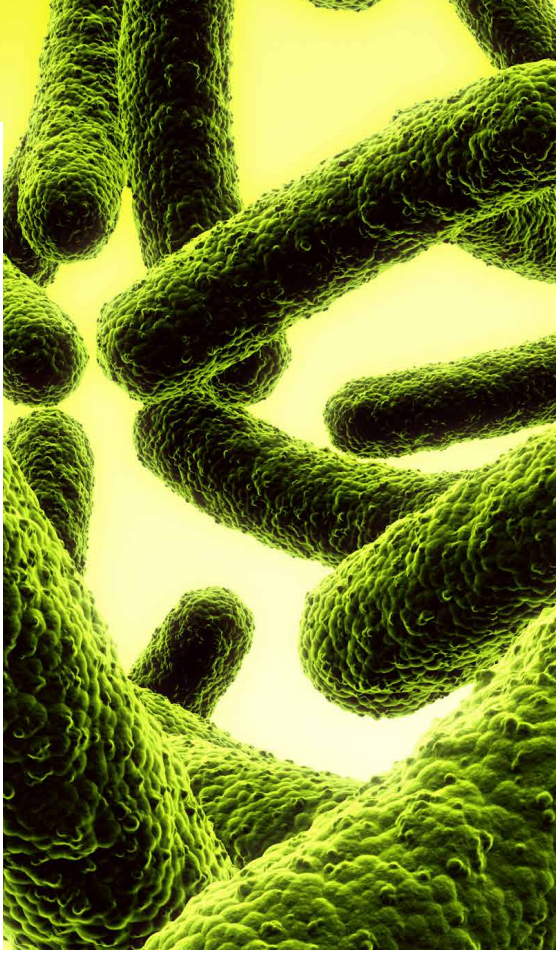
USDA United States Department of Agriculture National Institute of Food and Agriculture



UTILISING THE PLANT MICROBIOME IN AGRICULTURE

Dr Philippe Rolshausen and his team at the University of California, Riverside, aim to understand the microbial communities that live on and in fruit trees, with implications for crop yield and plant disease. With backing from Californian tree crop industries and funding from state and federal institutions, his group uses a suite of 21st century 'omics' technologies to delve into the mysteries of the plant 'microbiome' and develop farming practices to support plant health.

Fruit is considered an important component of a healthy diet. This assumption forms the basis of government guidelines, official health and dietary advice, conventional wisdom and food marketing. In recent years, the global fruit market has increased significantly. Global production of fresh fruits grew by 50% between 2004 and 2014, with 5.4 million hectares dedicated to cultivation in 2014, up from 3.8 million in 2004 (FAOStat, http://www.fao.org/faostat/). With 2 billion more people to feed by 2050, this trend will need to be sustained, and we will also need to find alternative ways to grow food. On the 'supply' side, this has been driven by advances in cultivation and processing technologies, globalisation and sophisticated global supply chaining, allowing on-demand availability of a wide diversity of fruits, all year round. On the 'demand' side, this has been driven by greater affluence and health consciousness of consumers, increased demand for healthier and organic food products, and changing consumer tastes. The production of fresh and processed fruit products is important to the economies of several countries, as well as the dietary health of billions.

Fruit cultivation for many invokes imagery of family-run farms, postcard scenes of idyllic vineyards and orchards, and grandparents' tales of Saturday afternoon apple scrumping escapades. While these images may bear some resemblance to fact, fruit farming and processing has become increasingly industrialised, with mechanisation and automation standard within this sector. However, even in the era of 21st century agribusiness, yield and quality in fruit production are very much dependent on the prevailing environmental conditions, both biotic and abiotic.

There is a complex web of biotic factors influencing crop cultivation that agriculturists must understand and control. As well as 'macroscopic' and easily observable organisms, such as birds and insects, invisible micron-scale organisms, so small that they cannot be detected without advanced microscopy, can also influence crop yields and quality (positively or negatively). Plants host complex communities of microorganisms, including bacteria, fungi and viruses, within their tissues (endophytes) and on their surfaces (epiphytes). This is known as the plant-associated microbiota, the plant microbiome or the phytobiome. We are only just beginning to scratch the surface of this invisible yet important world of plant-associated microbes, with important implications for crop production.

The human microbiome is a field of intense investigation, particularly the influence of human-associated microbes on disease, immunity, nutrition, health and wellbeing. This research has spawned the multi-billion dollar probiotics industry, worth over $36 billion in 2015, and projected to grow to $64 billion in 2023 (http://www.prnewswire.com/news-releases/probiotics-market-size-to-exceed-usd-64-billion-by-2023-global-market-insights-inc-578769201.html). While the health benefits of probiotic foods and supplements are still subject to scientific validation, the media and the internet often propagate misinformed half-

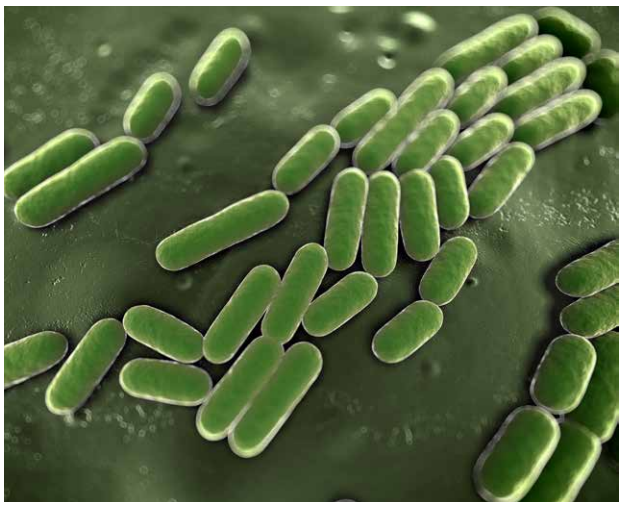
‘Just like for humans, I believe we can develop and commercialise probiotics for plants’

truths on their efficacy. In contrast, little such commercial or media attention is given to the plant microbiome for agriculture, despite its importance to global food production.

Dr Philippe Rolshausen, a researcher in the Department of Botany and Plant Sciences, University of California, Riverside, hopes to raise the profile of the microbiome in crop production. ‘Microbiome research is an exciting developing field that has received a lot of attention and publicity especially for human research, but for agriculture we are far behind,’ says Dr Rolshausen. Some microbes are demonstrably beneficial to the plant host, such as root-associated mycorrhizal fungi that sequester phosphates, nitrogen-fixing rhizobacteria, plant growth promoting rhizobacteria and fungi and bacteria that stimulate plant defences. The majority of microbes are considered to be commensals, deriving benefit from the host without causing obvious harm or help. A number of microbes are pathogenic and can severely impact crop productivity that can lead to devastating socio-economic outcomes. The most notorious example is the Irish potato famine caused by the soil-borne fungus *Phytophtora infestans*, the causal agent of the potato blight disease that occurred in Ireland in the mid-19th century https://www.britannica.com/event/Great-Famine-Irish-history. Dr Rolshausen works on economically detrimental vascular plant fungal and bacterial diseases affecting specifically grapevine and citrus. Only when such crop diseases are understood can effective strategies be implemented for their prevention and mitigation, and Dr Rolshausen’s group aims to elucidate the pathogenesis of these diseases on a microbial and molecular level.

Dr Rolshausen liaises closely with commodity groups, to carry out microbiome research mainly with tree crops – crops of particular significance to the Californian economy and culture. ‘Today, one of the most important problems that the California tree crop industries have to cope with are invasive pests and diseases,’ he explains. ‘I am working with these industries to develop and implement innovative yet practical solutions to address these challenges.’ Using a suite of 21st century ‘omics’ technologies, his group aims to elucidate tree crop microbiota, and use these insights for diagnostic purposes and to devise strategies to fight pathogens and boost yields. In particular, the group’s use of DNA profiling of host-associated microbial communities has led to some interesting and novel insights regarding the complex interactions between the microbiota, the plant host and the environment. It appears that host plants selectively recruit specific microbial species to colonise particular tissues, and the prevailing environmental conditions, (such as irrigation, fertilisers and pesticides application) can influence microbial colonisation and community dynamics.

Biotic stress factors (i.e., plant pathogens) greatly influence the host microbiota, a research topic of great significance to Dr Rolshausen and his team. One potential outcome of this research, which the group is keen to pursue, is the development of microbiota-based diagnostic tools for early detection of plant diseases, following on from their current focus on DNA-based pathogen diagnostics. In the framework of a project funded by the grape industry, and in collaboration with UC Davis, the team are investigating next generation (DNA) sequencing (NGS) approaches to identify and quantify fungal pathogens associated with grapevine trunk diseases. Trunk diseases are caused by a complex of fungal pathogens, and applying NGS technology will allow the accurate and early diagnosis of infection, identifying the fungal taxa responsible for the disease so that adapted management strategies can be implemented in a timely manner. Another yet more exciting outcome would be to promote the plant’s ‘friendly’ microbiota to confer tolerance and resistance against pathogens. The group hypothesises that some the plant-associated microbes



operate on a molecular level, producing natural antimicrobial compounds that inhibit the growth of pathogens. Furthermore, they hope to develop probiotic approaches to encourage the growth of symbiotic microbial communities to improve plant health. 'Just like for humans, I believe we can develop and commercialise probiotics and prebiotics for plants,' Dr Rolshausen explains.

Management of Pierce's Disease in Grapevine using a Microbiome Approach

Few natural phenomena strike the fear in the hearts of Californian winegrowers like Pierce's disease (PD). In the 1940s and 1990s, vineyards in southern California were decimated by PD, raising concerns among grape stakeholders. PD causes proliferation of diseased vines that leads to vine death in as fast as two years, reducing availability of high-quality grapes for winemaking. The disease is caused by *Xylella fastidiosa*, a bacterial pathogen that resides in the xylem vessels of affected plants. It spreads between plants by insect vectors, especially the native blue-green sharpshooter (*Graphocephala atropunctata*). However, the 1990s blight was spread mainly by an invasive species – the glassy-winged sharpshooter (*Homalodisca coagulata*). The problem in southern California was exacerbated by the rapid intensification of both grape and citrus industries, and citrus groves and vineyards in close proximity to each other. Citrus trees prove an ideal feeding and breeding host for *H. coagulata*, which experience a population explosion and then fly into neighbouring vineyards to feed on the vines. Up to now, the problem has been managed by intensive insecticides, increasing the risk of resistance among insect populations. Yet, this invasive insect vector has moved its way to northern California, at the epicentre of the wine grape industry.

Upon observation that certain grapevines in natural vineyard settings express reduced susceptibility to the disease, Dr Rolshausen's group is investigating the role of the microbiome associated with those specific vines. They carried out microbial community analysis and metagenomic screening to identify microbes that apparently confer tolerance to PD. These identified two bacteria, *Pseudomonas fluorescens* and *Achromobacter xylosoxidans*, that were associated with less PD symptoms in affected vineyards. The mode of action of the two bacteria is currently being investigated in plants. A number of microbes isolated from these vines were also found to inhibit *X. fastidiosa* growth in in vitro inhibition assays. Analysis of metabolites from one fungus, *Cochliobolus* sp., revealed that a small organic compound, radicinin, was the causative agent of antimicrobial activity. This is currently being developed into a formulation for downstream commercial curative applications.

Citrus Huanglongbing and the Search for New Control Strategies

Huanglongbing (HLB) is one of the most destructive diseases of citrus globally. It is caused by colonisation of the citrus tree's phloem vessels for sugar transport, by the bacterial pathogen *Candidatus* Liberibacter asiaticus (*C*Las), and is spread by the invasive insect vector, the Asian citrus psyllid (ACP), *Diaphorina citri*. Native to southern Asia, *D. citri* has spread worldwide, including the US, and has been particularly destructive to the citrus industries in Florida. The ACP vector is now established in California, where several HLB infected trees have been found.

*C*Las cannot be cultured, greatly hampering research into HLB. Despite this, Dr Rolshausen's group is using culture-independent DNA-based techniques to investigate the associations between *C*Las and citrus plants. Importantly, robust diagnostic tools are necessary for early detection of *C*Las, especially at the early stages of an epidemic like in California, so that infected trees that would otherwise serve as a *C*Las inoculum reservoir, can be removed early on. Conventional PCR-based methods are not always reliable, and can fail to detect *C*Las due to spotty distribution in trees, giving false-negatives. A number of more reliable alternative diagnostics are being developed, based on the biochemical changes to the host upon *C*Las infection, such as those that detect changes in volatiles emitted from tree foliage. Research teams are also using metagenomics and microbial community analysis of citrus tree tissues, to look at changes in the microbiome as a function of HLB infection. Thus, organisms associated with citrus trees may turn out to be promising biomarkers of *C*Las infection. Similar to PD in grapevines, some citrus trees appear to cope with the disease (a.k.a. survivor trees). Dr Rolshausen's team is mining this survivor tree phenotype in order to identify beneficial microbes and/or compounds that could confer increased tolerance to HLB.

Tree Crop Microbiomics: The Way Forward

Dr Rolshausen and his colleagues have shown that what is needed is a more holistic approach to tree crop agriculture. Crops must not be merely viewed as static, sterile biological production units. Indeed, it would be wrong to think of crop plants as 'just plants', but as hosts for a diverse and dynamic communities of microbes, constantly interacting with the host and the environment in interesting ways. Our knowledge of these fascinating and important biological interactions is still fairly limited, and the team's mission is to elucidate what constitutes a healthy plant microbiome and identify beneficial microbes that promote plant health and provide protection against biotic and abiotic stresses.

The economic significance of this research cannot be underestimated. As global tree crop farming expands and becomes ever more intensive, the threat of pathogenic plant diseases increase, especially when cultivation practices are not conducive to optimum plant health. There is a need for a deeper understanding of pathogenic plant disease, reliable diagnostics for early and quantitative detection, disease-fighting strategies based on the plant's natural microbiome-mediated 'immunity', and the implementation of such insights into routine tree crop management.

Meet the researcher

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Dr Philippe E. Rolshausen completed his BSc in Plant Biology at the University of Tours, France, in 1994, and went on to study for an MSc in Ampelography (Viticulture) at INRA, University of Bordeaux, France, in 1995. Being instilled with a passion and fascination of the science of winemaking, he went on to gain a PhD at the Department of Plant Pathology, University of California, Davis, in 2004. His dissertation topic was 'Biology and control of Eutypa dieback of grapevine in California'. Apart from a brief stint as a Post-doctoral Researcher at the University of Connecticut, he has held a number of extension, outreach and research posts at the University of California, almost continuously to the present day. His main research interest has been the biology and management of pathogenic diseases of grapevines, particularly in the context of the plant microbiome. He is now applying microbiomic approaches to other tree crops, including citrus, almond, pistachio and avocado.

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UC RIVERSIDE UNIVERSITY OF CALIFORNIA

ANIMAL SCIENCE

TOWARDS ETHICAL ANIMAL PRODUCTS

Globally, about 70 billion farm animals are farmed for meat, milk and eggs every year. That's almost 10 times the current human population. The vast majority of these animals are intensively farmed – being kept permanently indoors in cramped conditions to keep costs down – with devastating consequences for animal welfare.

Animal wellbeing has also suffered greatly due to selective breeding practices – where breeders have selected animals for their ability to rapidly gain weight, or produce more milk, for example. Although selecting for these traits has driven down the price of meat and dairy products, the animal welfare costs have been disastrous.

For instance, breeding chickens to produce more meat has given rise to the creation of 'broiler chickens' – birds whose bodies grow so quickly that their legs often buckle under the strain, causing them great pain and distress. Furthermore, the hearts and lungs of these birds often cannot keep pace with their overgrown bodies, and it has been estimated that hundreds of millions of chickens die from heart failure every year. Dairy cows have also suffered greatly due to selective breeding strategies, with many producing so much milk that they develop painful conditions such as mastitis.

Clearly, these breeding practices are far from sustainable – they not only pose serious animal welfare issues, but also lead to decreased food yields due to death and disease. In keeping with our sustainable agriculture theme, this section showcases the work of several researchers, who are developing effective solutions to our unethical treatment of livestock.

First, we meet Dr Macdonald Wick at Ohio State University, who studies muscle problems in chickens and turkeys that have been bred for meat production. Selective breeding for larger body weights has given rise to a high prevalence of breast muscle disorders (myopathies) in these birds, taking a large toll on their health and wellbeing. Dr Wick's group are working to understand the molecular mechanisms that underlie poultry muscle growth and development – using a combination of poultry husbandry, material science, genetics and biochemical tools. They hope that their research will lead to the establishment of biomarkers for future breeding strategies that balance animal welfare with the economics of meat products.

Next, we showcase the work of Dr Chad Dechow and his research team at Pennsylvania State University, who are particularly interested in the health and wellbeing of dairy cattle. As mentioned above, selective breeding for milk yields has had negative impacts on the health of dairy cows, with many of them being prone to mastitis and more susceptible to contracting pneumonia. Not only are these issues a serious welfare concern, but mastitis leads to milk that is unfit for human consumption, while the high death rate associated with pneumonia has huge economic consequences for farmers. By adopting a genetics approach, Dr Dechow and his colleagues are evaluating new selection and crossbreeding options to improve dairy cattle health. Through the breeding strategies that they are developing, the team plans to enhance traits such as disease resistance, which will also lead to a reduced need for antibiotics.

Bovine Leukaemia Virus (BLV) is another common pathogen that affects dairy cattle, and new evidence suggests that it may be more damaging to US cattle than had been previously thought. In the next article of this section, we feature the research of PhD candidate Meredith Frie and her colleagues at Michigan State University, who are working to understand how BLV impacts animal health, in the hope of curbing the spread of the virus for happier, healthier cattle.

Of course, viruses and bacteria are not the only pathogens that affect domesticated animals – intestinal worms can lead to immense suffering, due to poor nutrient absorption, anaemia, muscular weakness, impaired immune function and intestinal blockages. Here's where the research of Dr Paul Slusarewicz and his team at MEP Equine Solutions comes in, who we'll meet in the next article of this section. These innovative researchers have revolutionised the way that veterinarians detect and quantify worm infections, allowing them to use their smartphones to count the number of parasitic eggs in faecal samples. With the team's new technology, a procedure that once took days to produce results can now be performed with surprising accuracy in less five minutes out in the field. Rather than

applying monthly rotations of de-wormers to their herds, horse owners and farmers can now provide targeted treatment that fully addresses the unique needs of a specific animal. Such strategic application of de-wormers in infected animals will reduce the rates of drug resistance in intestinal worms.

As well as disease and the health problems associated with selective breeding, keeping farm animals confined is another serious welfare concern. In fact, the vast majority of animals that humans raise for meat are kept indoors in extremely cramped conditions, causing them great distress. Furthermore, keeping animals indoors in such close proximity to each other is an ideal environment for deadly pathogens. To ward off bacterial pathogens, around half of the world's antibiotics are fed to farm animals, leading to antibiotic resistance.

For the reasons stated above, many of us are looking for alternative sources of meat – from animals that are not intensively farmed. One option is meat obtained from one of the most ancient of human activities – hunting. Indeed, meat obtained from hunting wild animals can form an important component of people's diets, reducing our reliance on intensive farming practices. In the next article, we meet Dr Shawn Riley and Dr Göran Ericsson, who study the sociology of wild-harvested meat. The team works to understand the distribution of wild-harvested meat, how this meat moves through society, and the factors that influence people's perception of hunting.

And of course, animal welfare and health are not the only important considerations when it comes to meat – another big concern is food safety. In fact, meat and other animal products are responsible for the majority of food poisoning cases.

To tackle this issue, a group of food scientists decided to gear their research efforts towards improving the safety of meat and poultry, and formed a collaboration known as the Consortium of Food Process Validation Experts. As part of this important collaboration, the scientists involved are advocating the use of non-pathogenic bacterial substitutes to simulate the pathogen of interest. In the final article of this section, we detail the team's novel approach to food safety validation.

ILLUMINATING THE CAUSES BEHIND MUSCLE PROBLEMS IN POULTRY

Muscle defects and non-infectious anomalies in commercial poultry are increasing in prevalence – with implications for bird welfare, meat quality and economic value. **Dr Macdonald Wick** and his team at Ohio State University are attempting to elucidate the biological mechanisms underlying these defects – as well as their genetic and environmental basis.

Poultry meat is now a huge part of our diets in the developed world – chicken for Sunday roasts and midweek meals, turkey for Thanksgiving or Christmas, takeaway fried chicken, not to mention a plethora of processed poultry products. It's hard to imagine that in our great-grandparents' day, chicken was an expensive treat reserved for special occasions. During the difficult years of WWII, consumption of chicken boomed in America, and the post-war 'Chicken of the Future' contests of the late 1940s were launched by the US poultry industry to ensure that this trend would continue.

This set in motion the decades-long program of commercial poultry production that continues to this day. While this intensification of poultry farming is epitomised with an increase in large poultry production complexes where every aspect of raising the birds is controlled under optimised conditions, perhaps the biggest change is in the birds themselves. Decades of selective breeding has changed the genetics of chickens and turkeys, with poultry breeders selecting for faster growth and ever increasing body weights. This has resulted in the generation of 'broiler' chickens – large chickens favoured for meat production – which are distinct from 'layers' for egg production.

These innovations in breeding and production have contributed to satisfying our palates with affordable and plentiful poultry, and our demand for poultry continues to grow. In 2016, over 15 million metric tons of broiler meat were consumed in the US, and nearly 90 million tons worldwide, and this is projected to increase even further (https://apps.fas.usda.gov/psdonline/circulars/livestock_poultry.pdf). However, this has not been without costs – both for the birds themselves and consumers.

Poultry, particularly chicken and turkey, is typically considered as 'white meat'. More specifically, it is the breast and wings that are considered 'white meat', while the drumsticks and thigh are regarded as 'dark meat' – based on differences in appearance, taste and texture. These gastronomic distinctions are actually based on the biology of the birds and are largely due to the energy requirements and respiratory oxygen demands of the muscles. The birds' legs are highly vascularised, composed of 'slow twitch' muscle fibres that are rich in mitochondria (the sites of aerobic respiration) and myoglobin (temporary repositories of oxygen), as standing and walking require continuous work for long, sustained periods.

In contrast, the breast is poorly vascularised and is made up of two distinct pectoralis muscles with far less myoglobin – the pectoralis minor or tender and the more prevalent pectoralis major. The pectoralis minor muscles are responsible for raising the wings during flight and the larger more powerful pectoralis major muscles – composed of 'fast-twitch' muscle fibres – are needed for pulling the muscles down. The pectoralis major deploys an oxygen free – or 'anaerobic' – type of respiration to meet the intense, periodic energy demands of strenuous activity – perfect for flight in the wild ancestors of domesticated poultry but not for the confinement poultry rearing associated with commercial production systems.

Selective breeding for a heavier body weight and increased proportion of breast muscle, or breast yield, has resulted in increased muscle accretion but has taken its toll on muscle quality and the consequential health and welfare of the birds. A worrying, yet not uncommon, bout of breast muscle disorders – or myopathies – have been occurring in commercial broilers and turkeys in recent years. As far back as 1968, muscle myopathies were observed in turkeys, just as confinement poultry rearing was becoming mainstream. In the ensuing decades, these myopathies have become more common, and today, the predominant myopathies observed in broilers are known as 'white striping', 'woody breast' and 'pale, soft, exudative' (PSE) meat. 'These myopathies present a challenge to both the welfare of the animal as well as economic challenges to the poultry industry,' says Dr Macdonald Wick, of Ohio State University.

Genetic and Environmental Factors Behind Poultry Myopathies

Since the first reports of muscle myopathies in the 1960s, the underlying physiology and aetiology of these anomalies in poultry, and how to avoid them, have been of great interest to producers, breeders and poultry scientists. Despite the welfare and economic significance, much of the research to date has focused on comparing abnormal and normal tissues from older birds with few studies on the developmental progression of these disorders. Moreover, much of the research to date has been fairly descriptive and often involves histological studies – involving examination of tissues under the microscope. While such studies have been useful, they have provided little insight into how or why poultry muscle disorders develop.

Dr Wick and his research team at Ohio State University are hoping to change this. The team includes Dr Jacqueline Griffin, Dr Mike Lilburn, and statistician Dr Eduardo Luis Ferraz Dias de Moraes – who provides much needed statistical analysis to the team's studies. Dr Wick's group aims to elucidate the molecular mechanisms underlying poultry muscle growth and development – using a combination of poultry husbandry, material science approaches for studying meat, biochemical tools – and increasingly, cutting-edge 'omics' tools.

'We hypothesise that temporal changes in these mechanisms are integral to muscle health and post-mortem meat quality,' says Dr Wick. The team is particularly interested in the various influences – both genetic and environmental – that affect poultry muscle development, as well as the post-slaughter conditions that directly affect meat quality. The team has extensively studied the influence of bird genotype on muscle development – with particular emphasis on meat processing characteristics.

As mentioned earlier, a particular concern for the poultry industry is a meat quality issue known as 'pale, soft, exudative' (PSE) meat. While still nutritious, PSE poultry has properties that consumers may regard as aesthetically unappealing, notably reduced water-holding capacity resulting in a dry texture and a reduced propensity for gel formation. PSE meat is also a problem for food processing and preparation, as the loss of moisture during cooking requires repackaging and increased labour costs. Worryingly, it is difficult to differentiate between normal and defective breast tissue immediately after slaughter, and very often this is only apparent after cooking, when meat products appear hard and dry. It is estimated that the incidence of PSE in turkey meat approaches 20%.

Several factors are thought to influence the development of PSE meat – including genetics, adverse rearing conditions such as heat stress, pre-slaughter anxiety, and post-slaughter pH and heat conditions. The breast muscles still undergo metabolism for a short time after slaughter, and being composed of 'fast-twitch' muscle fibres, are capable of undergoing anaerobic respiration after respiration stops. During post-slaughter metabolism, glycogen (the primary carbohydrate energy store) is rapidly broken down into lactic acid in the absence of oxygen, accompanied with a rapid drop in pH. Dr Wick's team is trying to shed light on this process – and they are particularly interested in how the proteins present (the 'proteome') in breast muscle may stimulate or impede this acidification.

The research group has meticulously studied the various factors that lead to PSE meat in turkeys. For these studies, they use breast muscle from turkeys hatched, raised and processed at research farms under carefully controlled conditions with the muscle/meat being subjected to a battery of tests. These studies typically involve comparing breast muscle from three turkey strains – one representing commercial turkeys from the 1960s (RBC2), one selected for body weight (F-line) and a fast-growing commercial strain with enhanced breast muscle yield (C-line). After slaughter at 16 weeks, breast

muscle is harvested and the team analyses its water-holding capacity, gel formation, elasticity and biochemistry. The team's studies have provided insight into the incidence of PSE meat in poultry and found that breeds selected for body weight and breast yield produced breast meat with decreased meat functionality. In particular, the water holding capacity of the C-line breed was significantly less than that of RBC2, with that of the F-line being somewhere between the two (Updike et al., 2006).

It is known that the predominant protein present in breast muscle meat, known as myosin, is a major factor influencing meat gel formation – and thus, may be used as one indicator for PSE meat. Although poultry lines with increased body weight or breast yield are shown to have a greater susceptibility to PSE and other muscle quality issues, studies by the team seem to suggest that levels of myosin are comparable between the different turkey strains. Indeed, one of their studies suggests that other proteins are also involved in gel formation and product quality problems may be due to the balance of various proteins that accompany the growth of pectoralis major muscles.

Notably, Dr Wick's group found that feeding regimes can affect the expression of neonatal and adult fast skeletal muscle myosin proteins in F-line and RBC2-line young turkeys. These proteins are sequentially expressed and may be indicators of developmental progression in muscle. The turkeys were fed the Ohio State University turkey starter diet, but there was a twist – they were split into two groups – some given unlimited access to food while others were restricted access to food for 30 minutes daily. Subsequently, the birds were euthanised and the group measured the ratios of neonatal and adult myosin in breast meat samples. In F-line and RCB2-line turkeys, proportion of adult fast skeletal muscle myosin protein was significantly higher in those that had unlimited access to food.

'Transcriptomics' for Elucidating Myogenesis in the Egg

Muscle development – or 'myogenesis' – is a complex yet tightly regulated temporal and spatially specific process in poultry that begins with the embryo while still in the egg. Precursors to muscle cells, known as myoblasts, migrate and fuse together to form fibres known as myotubes, which subsequently differentiate into mature muscle fibres.

There are at least six different developmental types of fast myosin, and the expression levels of these different types can be reflective of the embryonic and post-hatch development and myogenesis. Myogenesis doesn't stop in the egg though! After hatching, myogenesis continues as the muscle cells expand. A recent focus of the Dr Wick's research has been elucidating the mechanisms behind embryonic myogenesis – a little-understood yet critical stage in meat production. In this line of research, his team is using cutting-edge transcriptomics tools to study the expression of proteins involved in myogenesis, using the temporal expression of the myosin isoforms as a developmental gauge with embryos of the single-comb white leghorn chicken as a model organism and broilers.

The instructions for protein expression are transferred from DNA to mRNA 'transcripts' in a process called 'transcription' and are subsequently used to build new proteins in a process known as 'translation'. Using transcriptomics to quantify specific mRNA transcripts, the team can elucidate which transcripts are being expressed, and by how much.

The team is currently validating the use of a transcriptomics platform – NanoString nCounter Transcript Abundance Analysis – to understand embryonic myogenesis on the transcriptional level – with promising preliminary results (Griffin et al. 2016). The researchers compared mRNA transcript levels corresponding to key proteins in embryos of white leghorn chickens with another broiler breed that exhibits partial muscular dystrophy. They found that differences in transcription profiles for myogenesis-related proteins that later dictate muscle fate in hatchlings are already present in the embryo.

With the publication of a novel muscle myopathy staging method (Griffin et al. 2017), the team is validating the transcriptomics platform in the quantification of transcription in broiler chickens every other day post-hatch to elucidate the myogenic signals and proteins associated with the progression of white stripping and woody breast.

Summary and Future Directions

The insatiable demand for inexpensive, affordable poultry meat has pushed chickens and turkeys to their physiological limits – impacting the welfare and muscular health of the birds, as well as the quality of their meat. Dr Wick and his team are unravelling the biological mechanisms behind muscle problems in poultry, and are pioneering 'omics'-based approaches to illuminate the genetics behind myogenesis and myopathies. Their work will eventually lead to the establishment of biomarkers for future breeding strategies that balance welfare with the economic value of meat products.

Meet the researcher

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Dr Macdonald Wick gained his PhD in Agricultural Chemistry at University of California in 1997 after having graduated with a BS in Microbiology at California State Polytechnic University in 1976. He is currently an Associate Professor at the Department of Animal Sciences. A prominent researcher in the field of meat science, Dr Wick's special interests include muscle growth, the genetic, environmental and postmortem impacts on meat quality and thermally-induced meat gel formation. Over the course of his career, he has received a number of high-profile Research Enhancement Competitive Grants from the Ohio Agricultural Research and Development Center to investigate poultry meat quality and nutritional strategies. Dr Wick is keen to disseminate his research findings to industry, and regularly interacts and collaborates with meat processing companies. He also teaches a number of Animal Science and Meat Science modules for undergraduate students, including Animal Laboratory Techniques and a freshman research seminar series.

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THE OHIO STATE UNIVERSITY

THE DAIRY COW: BEYOND MASS PRODUCTION

As dairy farmers struggle to make ends meet, economic efficiency often forces the health and wellbeing of cattle to take a back seat. **Dr Chad Dechow** and his team at Pennsylvania State University have set about tackling this problem, providing a means for producing healthy, fertile cows, without compromising on productivity.

As the global human population continues to soar and multinational economics drive our societies, dairy farming is witnessing an era of tremendous growth and change. An increased demand for dairy products coupled with tight financial margins has resulted in the large-scale expansion and intensification of the farming industry. That quaint, family farm you used to visit at the bottom of the village is struggling to stay in business, as we make way for high-yield, intensive production systems – with each cow representing a single cog in this vast network of mass-production.

Cattle farmers across the globe strive to stay on top, encouraging their herds to metamorphose through selective breeding to achieve the highest productive efficiencies of milk, fat, and protein. 'We have made a lot of changes to dairy cows over the last half-century – milk production levels have doubled for instance, largely because of genetic change,' says Dr Chad Dechow of Pennsylvania State University. 'This has many benefits as we are able to feed a growing population more efficiently.'

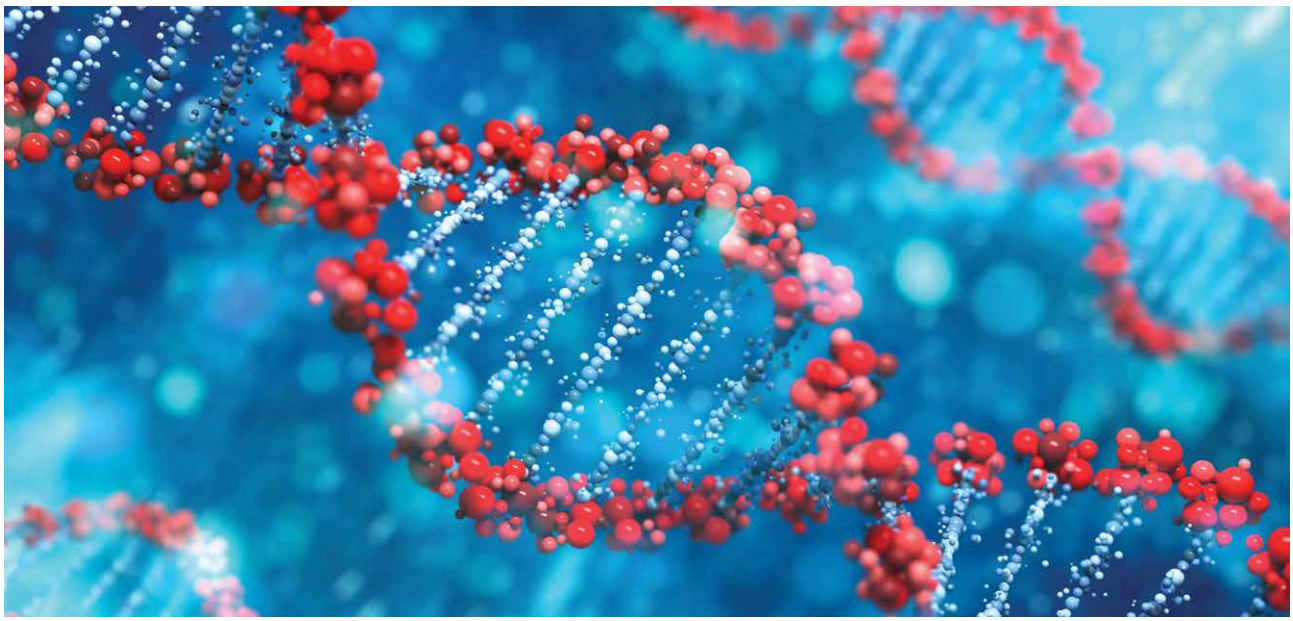
However, with this emphasis on improving economic efficiency, breeders weren't always encouraged to select traits relating to the health and fertility of cattle. Although some anticipated adverse effects on cattle fitness and mortality, a look back at historic trends can be eye opening. Since selective breeding accelerated with the widespread adoption of artificial insemination in the 1970s, declines in cattle health and fertility, with increasing incidences of premature death and lameness, have been observed. Not all, or even most, unfavourable trends are due to genetic changes in our cows.

Mastitis and pneumonia are two examples of common diseases in dairy cattle, and both have a huge effect on the annual yields of dairy farms around the world. Mastitis-induced inflammation of the udders leads to milk that is low in potassium, calcium, and casein – the main protein found in milk. Not only does mastitis result in low-quality liquid that is unfit for sale, but the resultant infections cause udder damage and are occasionally fatal. Mastitis is still the most costly disease to the dairy industry, but through better herd management and selection for traits such as udder conformation and disease resistance, mastitis is one disease that is actually less prevalent now despite higher milk yield.

Rising concern for animal welfare is one factor that has led to growing success in the organic farming industry. These dairy farmers, however, have their own unique challenges – although providing a more natural environment appears to benefit their cows' health and wellbeing, the lack of antibiotics means that many easily treatable conditions cannot be kept at bay. While conventional farmers use antibiotics to treat infectious disease, their organic counterparts must instead be proactive, adopting preventative measures to avoid contracting the bug in the first place. Pneumonia can be a particularly big challenge – it is highly contagious in young calves and without the help of antibiotics, a calf's lungs can be permanently damaged.

Dr Dechow, who actually grew up on a dairy farm, leads studies that are focused on remedying this problem. His team aims to provide a means to monitor and overcome prolific diseases for organic and non-organic farmers alike. He tackles the problem from a genomics approach, arguing that, 'genetic selection can be part of the solution'.

With the consumption of organic produce becoming more popular, and greater pressures on conventional farmers to decrease the prophylactic use of antibiotics, providing cattle with superior all-round genetics is key for farmers of the future.



Genetics and Genotyping

As international champions of milk production, the Dutch-built Holstein Friesians are the most prevalent breed of dairy cattle. Dr Dechow tells us that he and his team are 'beginning to evaluate genetic selection and crossbreeding options to improve calf and cow health in organic dairy herds', in collaboration with researchers from the University of Minnesota, Alfred State College and USDA – ARS.

Much of this work focuses on a mix of Holstein and crossbred herds. The researchers have access to commercial organic dairy farms throughout the US, which they can compare to two experimental herds. On the commercial farms there are pure Holsteins and crosses with various breeds including Jersey, Norwegian Red, Normande, Swedish Reds and Montbeliardes.

In this ongoing project, Dr Dechow and his colleagues are currently analysing the health and welfare of cattle on all farms, and aim to compile this information on a standardised database. Although measuring animal welfare can prove difficult, as there are no obvious quantitative measures, the team is implementing the following methods.

They individually genotype (analyse the genomes of) each cow and calf on the farms, using hair samples as genetic material. By analysing genetic variants known as single-nucleotide polymorphisms (SNPs), they are able to predict genetic merit for specific traits, such as resistance to pneumonia. SNPs are points on the genome that vary between individuals, and these differences can provide the team with information regarding the expected health and behaviour of the cows.

Further, Dr Dechow and his colleagues use their genotyping data to distinguish between cows that possess genes coding for the protein A1 beta-casein and those with genes for A2 beta-casein. These two forms of the predominant protein in milk may have different effects on human health, with A1 beta-casein thought by some to have a negative effect on human gastrointestinal health. Research has not yet been extended to explore the effect of this protein on calves during weaning – if the team finds an association between A1 beta-casein genes in cows and their calves' gastrointestinal health, they can then select against this trait when breeding.

To measure calf health and wellbeing, the team weigh each individual, assign a respiratory score relating to presence of a cough or nasal discharge, and also assign a score related to the calf's faeces. Cow examination, on the other hand, involves scoring their body condition, movement, hygiene, lesions, and stature. The researchers also record the cows' white blood cell counts and how low their udders hang, as these factors correlate directly to mastitis.

The Tale of the Telomere

In another research direction, Dr Dechow and his team have been focusing on genetic components known as 'telomeres'. Every living organism on Earth possesses a genome, which consists of a number of pairs of large genetic bundles known as chromosomes (humans possess 23 pairs of chromosomes, while cows have 30). Chromosomes are rod-like structures that contain many thousands of individual genes. The tips of these rods, however, are particularly susceptible to becoming degraded every time they are replicated during cell division.

To avoid our genomes being eaten away over time, we have structures called 'telomeres' at either end of each chromosomal rod, to protect the vital genetic information within. When telomeres become degraded beyond a specific threshold, the cell they exist in dies – a process that occurs more commonly as we age.

Previous studies have shown that, in humans, the original length of our telomeres is inherited – and inherent short telomeres have been associated with diseases such as diabetes, renal failure and neurodegeneration. The degradation of human telomeres over time has also been shown to occur more rapidly under stress and disease.

Although little relevant research exists concerning telomeres in livestock, Dr Dechow's team showed an association between telomere length and cow health and premature death in a study they published in 2012. Since then, there have also been a few studies reporting a correlation between telomere length and milk yield in Holstein cattle.

Based on this previous research in both humans and cattle, the research team believes that the degradation rate of telomeres in cattle might offer some insight into animal welfare, providing them with a quantifiable element to measure. Genetic evaluation of telomere length in Holsteins could also provide a marker to help the team select for inherently healthy cattle with higher stress-coping abilities.

In one of their ongoing research projects, Dr Dechow's team implements a routine genetic procedure called qPCR to accurately measure telomere length. To scientifically quantify animal welfare, the team considers the health and stress levels of individual cows. When considering health, they examine blood samples, white blood cell counts, and activity levels, while the cortisol concentration in the cows' hair is used as a measure of stress. Cortisol is a well-studied stress hormone that can be easily extracted from hair, saliva and faeces – with hair providing a better indicator of more long-term stress.

Using these techniques, the research team aims to prove that telomere length is heritable and is an indicator of disease susceptibility and productivity levels, by comparing calves' telomeres with those from their mothers. This information will provide an invaluable guide for genetic selection. Furthermore, if they find a link between stress and disease levels and telomere degradation rates, the team will be able to confirm their hypothesis that telomere length is an indicator of cow health and wellbeing.

A Moove Towards Diversity

Intense genetic selection can also compromise the genetic diversity of cattle worldwide. Breeders have focused on artificial insemination from bulls of limited genetic lineages, based largely on the productivity and appearance of their daughters. Inbreeding levels have consequently risen, while bulls with unique lineages but lower daughter performance are cast aside, unable to pass their genes on to future generations. Rising inbreeding means that cattle become increasingly susceptible to genetic defects over time.

Dr Dechow is part of a research effort, in partnership with Dr Harvey Blackburn from the National Animal Germplasm Program repository, and Dr Wansheng Liu from Penn State University, to bring back the genetic variation that has been lost from cattle breeds such as Holstein and Jersey.

The research team has already determined that most Holstein bulls worldwide can be traced to one of two ancestral lineages, each dating back to 1880. Jersey bulls follow an even more drastic trend, with every individual originating from a single bull from 1898. This lack of genetic diversity is thought to be due to the boom in popularity of artificial insemination over the last 50 years, allowing thousands of progeny from a single bull, coupled with intensive selection for high-yield traits. These intensive, selective breeding programs have neglected any conservation of genetic diversity, particularly on the Y chromosome (the part of the genome exclusively passed from father to son).

Dr Dechow, Dr Blackburn and Dr Liu's most recent accomplishment includes the regeneration of two male genetic lineage of Holstein cattle, which had previously been lost from dairy farming altogether.

The team aims to generate a method for assessing the variation and fertility of each bull's Y chromosome, as well as checking whether regenerating genes that have been lost is actually beneficial in the first place. Not only is their work anticipated to provide valuable information about a rare lineage found in the National Animal Germplasm Program repository, but potential long-term prospects include the reintroduction of genetic variation back into commercially farmed cattle.

What's Next?

Dr Dechow and his team seek to improve the welfare of farmed cattle, by using their acquired information to develop best genetic selection and crossbreeding strategies. By enhancing traits such as disease resistance, organic farmers will benefit from this research, but conventional farmers will reap the rewards too. As Dr Dechow points out: 'Pressure to reduce antibiotic usage on conventional dairy farms is increasing, so the development of cattle with higher innate disease resistance is relevant to all farmers.'

Through their research and outreach efforts, Dr Dechow's team is on track to make serious advancements regarding the sustainability of dairy farms, and the health and happiness of dairy herds across the globe. Dairy cattle genetics researchers around the world, including Dr Dechow and his team at the Pennsylvania State University, have set about tackling this problem, providing a means for producing healthy, fertile cows, without compromising on productivity. Long gone are the days of selecting cows entirely for their milk production capacity.

Dr Dechow (right) and his research team with calves from a regenerated male lineage. CREDIT: Amy Duke, Penn State College of Agricultural Science

Meet the researcher

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Dr Dechow grew up on a small dairy farm in New York State that milked Friesians and a few Brown Swiss. After receiving a BSc at Cornell University and an MSc at Pennsylvania State University, he went on to achieve his PhD in Animal Science from the University of Tennessee in 2003. After this, he moved back to Pennsylvania State University, where he is currently an Associate Professor of dairy cattle genetics. Here, his research involves improving the health and wellbeing of dairy cattle, with a particular focus on organic farms. Dr Dechow's mission involves optimising the productivity and yield on these farms, whilst also maintaining a very high standard of animal welfare. Dr Dechow also teaches in the areas of animal genetics, dairy cattle selection, dairy herd management and the use of dairy management software. He also co-advises Penn State's Dairy Science Club and is the coach of Pennsylvania's 4-H Dairy Judging team.

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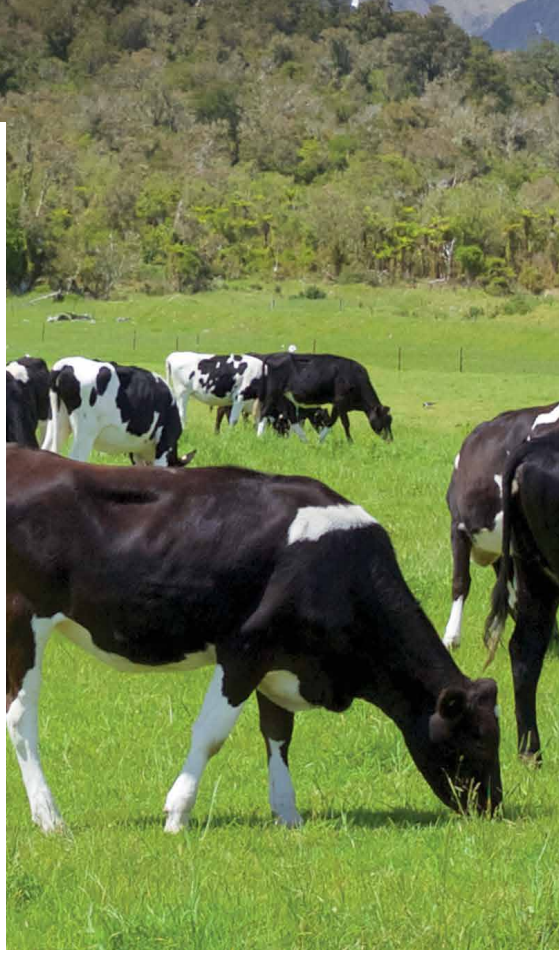
PennState

A HIDDEN THREAT TO HERD HEALTH: EVIDENCE FOR THE DAMAGING EFFECTS OF BOVINE LEUKAEMIA VIRUS

Diseases of agricultural animals are of major concern to people around the world, and thus a principle focus of many research programs. Research on the Bovine Leukaemia Virus (BLV) has been often overshadowed by other diseases that affect cattle, but new evidence suggests that the virus may be more damaging to US cattle than anyone had initially realised. PhD candidate **Meredith Frie** and colleagues at Michigan State University are working to understand how BLV impacts animal health, in the hope of curbing the spread of the virus for happier, healthier cattle.



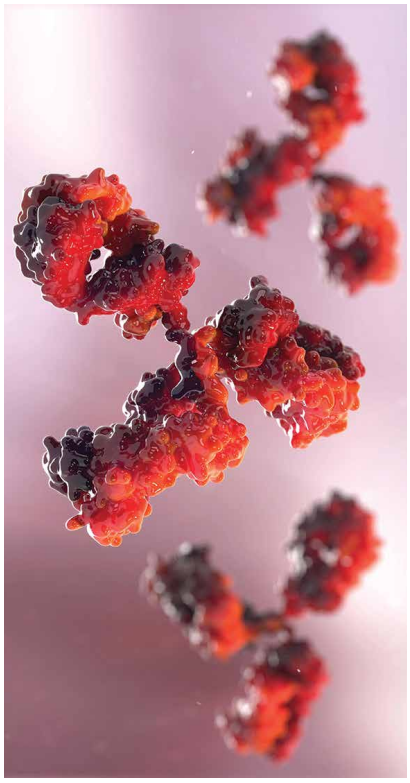
Flying Under the Health Radar

Worldwide, diseases that afflict agricultural animals cause massive economic losses and present huge barriers to animal health and welfare. Bovine Leukaemia Virus (BLV) is a common virus of cattle that causes lymphoma tumours in roughly 5% of the animals that contract the virus. These tumours can appear many places in the body and may account for nearly 14% of beef cattle and 27% of dairy cattle meat rejections. Although tumours only occur in a low number of infected animals, approximately 30% of infected cattle experience persistent lymphocytosis – characterised by extremely high numbers of lymphocytes circulating in the blood – a symptom that is commonly associated with impaired immune function.

The virus was only discovered in the late 1970s and is still poorly understood. It is known that the virus can be transmitted through bodily fluids, and the most common way it is spread may be during procedures where hypodermic needles or exam gloves are reused on multiple cows, along with dehorning, tattooing, fly bites, and young calves consuming milk from infected mothers. It is still unclear whether or not the virus may be transmitted during breeding or other common farm activities. While the most obvious effects of the disease have been noted in cows with tumours, the more hidden symptoms of BLV have only just begun to come to light through recent research efforts.

BLV has largely been eradicated throughout most of Europe following aggressive measures taken by 21 countries to eliminate the virus, through strict testing and culling of infected animals. However, millions of animals in the United States are infected – at least 83% of all US dairy cattle herds contain at least one infected individual, with infection rates within a herd typically ranging from 25 to 50%. Given these rates, it is predicted that nearly 40% of all dairy cattle in the US are infected with the virus.

Since its discovery, BLV has largely been ignored in the US due to the initial belief that the virus seldom causes disease, as lymphoma occurs in such a small number of cases. Despite the low rate of cancer occurrence, there is a serious economic cost of the virus – studies by Michigan State University's BLV research team suggest that approximately $520 million dollars are lost in the US due reduced milk production and shorter life spans of BLV-infected cows. Recent work by PhD candidate Meredith Frie and her thesis advisor Dr Paul Coussens in the BLV research team indicates that the virus may have additional health consequences beyond the potential for cancer, particularly reduced immune function, suggesting the need for control measures for the disease in the United States.



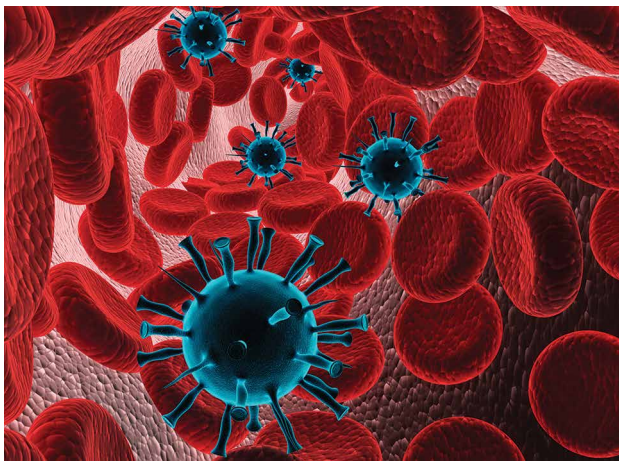
That is the real, ultimate goal of all of this research – to be able to contribute to a positive change in the industry that leads to reduced BLV infections and, hopefully, happier, healthier and more productive dairy cows.'

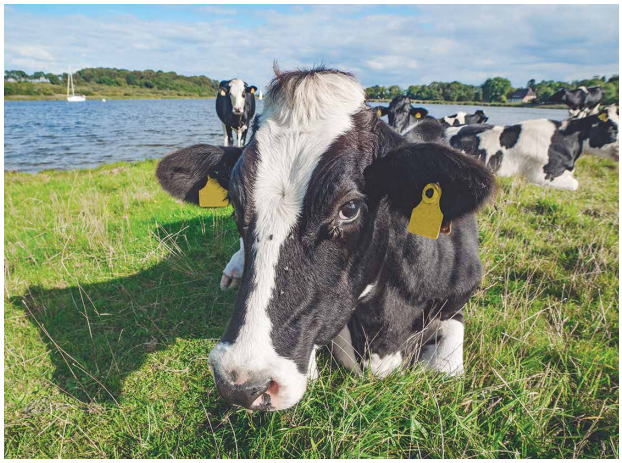
Adaptive Immunity and Herd Health

The mammalian immune system is composed of two parts – the innate immune response and the adaptive immune response. The innate response is our first line of defence, helping the body close wounds and remove debris. The adaptive immune response is our more specialised response that we build throughout our lifetimes, which learns to recognise diseases we come in contact with and builds customised defences called antibodies, in case the body ever encounters the same disease again. This adaptive response is the reason why vaccines work so effectively, as they activate the adaptive immune response and allow the body to form antibodies to components of a virus or bacteria, without requiring the individual to suffer through a full-blown infection. Once the adaptive immune system has formed an antibody for a particular pathogen, it more readily recognises it when exposed to it again, and is able to more quickly eliminate the threat than it would without the prior exposure.

Antibodies are essential components of the adaptive immune system. When the adaptive immune system is suppressed, such as in human patients with HIV/AIDS, individuals are highly susceptible to serious infections that an individual with a healthy immune system would easily clear. Frie and Coussens predicted that, beyond occasional cancer cases, the unmeasured cost of BLV was reduced adaptive immune function in infected animals. This effect of the virus could help explain why infected cattle with no cancer symptoms still produce less milk and live shorter lives. 'BLV is a close relative to HIV and we think it might pose a similar risk in terms of cattle health,' Frie explains. 'We suspect that BLV interferes with the immune system and makes BLV-infected cows more at risk for getting other infections that can be really detrimental to their health and their ability to produce high quality milk.'

Just as in humans, when immune function is suppressed in a cow, they are likely to be more susceptible to other diseases and BLV-infected herds spread destructive illnesses more quickly, with greater impact to animal health, wellbeing, and milk production. Further, it has been advised that BLV-infected cattle are less responsive to vaccinations and may not develop the same full immunity as a healthy cow when a vaccine is administered. While recent laboratory work with immune cells isolated from the blood of BLV-infected cattle has suggested that the components of their immune system demonstrate abnormal behaviour in response to pathogens, the immune response of BLV-infected animals has not been measured in live cattle in real





world conditions. Thus, Frie and Coussens set out to test if infected cows mounted a less robust adaptive immune response than healthy cattle when exposed to common immune challenges, with funding from the USDA, Michigan Alliance for Animal Agriculture and the Michigan Milk Producers Association.

Quantifying the Threat

In her first experiment, Frie examined the adaptive immune response of both BLV-positive and BLV-negative adult dairy cows when given a standard vaccination booster. Dairy cows are given a wide range of vaccinations to ensure that diseases are not able to easily spread through the herd. Frie and her colleagues, particularly Dr Kelly Sporer, looked at the response to a common multiple pathogen cattle vaccine that contains five viral strains and five bacterial strains, and is typically given at multiple time points throughout a cow's life. The cows in the study had already received initial vaccinations at a young age and were observed receiving a regular interval booster after at least six months had passed since their last dose.

The team collected blood samples the day before vaccination, and at days 7, 14 and 28 following vaccination, in order to observe how levels of adaptive immune system indicators fluctuated in response to the booster. From the blood, they isolated white blood cells and antibodies associated with the diseases present in the vaccine. They found that in BLV-infected cows, there was diminished white blood cell activity, and when these cells were cultured in the laboratory they showed abnormal responses to stimulation. BLV-infected cows also showed much lower levels of antibodies specific to the pathogens in the vaccine, indicating that their adaptive immune system had not responded as well to the vaccination as healthy cows. The results of this study strongly support the idea that BLV-infection impairs the adaptive immune system's ability to mount an appropriate response to a vaccine.

In their first study, Frie and her colleagues demonstrated how BLV-infected cows responded to a familiar immune challenge – a vaccine they had received multiple times prior to the study. In the second experiment, they wanted to observe how the adaptive immune system of BLV-infected cattle responds to a completely novel immune challenge, and how the response changes with a second exposure. For this study, the team needed to create an immune response to something the cows had never encountered before, but that would not be dangerous and degrade their long-term health. They chose keyhole limpet hemocyanin (KLH) – a protein collected from sea creatures that the mammalian body responds to in a similar way as it would to an actual pathogen by producing antibodies, and that a Midwest dairy cow would never have encountered in its regular life.

Frie and her colleagues injected 10 BLV-infected and 10 BLV-negative cows with KLH and observed their immune response over the next 28 days. Fifty-six days after the first exposure, they injected the same cows with KLH again and observed their response for another 28 days. The team found that following both the initial and secondary exposure, BLV-infected cows had lower levels of white blood cells and lower levels of KLH-specific antibodies. They also discovered that these white blood cells performed abnormally when cultured in the laboratory. Further, the viral load of BLV in infected cows increased following the exposures, indicating that the virus took advantage of the immune system fighting the KLH for its own gain. These results provide further evidence that BLV significantly impairs the immune systems of infected animals.

A Path Forward for Healthier Herds

When BLV was discovered in the late 70s, researchers in the US agreed that the disease posed little risk to the cattle industry, given the low occurrence of cancer in response to infection. At that time, only about 10% of the US cattle population was afflicted and it was decided that measures to control the disease were not necessary.

Presently, however, BLV afflicts approximately 40% of all US dairy cattle at a great economic and animal welfare burden. Frie's research indicates that the true burden of the disease may not even be fully appreciated, as infected cattle are likely to die from a different disease that they were only susceptible to due to concurrent BLV infection.

With such high national infection rates in the US, it is not realistic to take aggressive action against the disease, such as euthanising infected animals, but steps must be taken to reduce its spread in the hope of one day eradicating BLV, as has been successful in many countries across Europe, Africa and Asia. Practical measures that have been identified in the US include changing needles and gloves between animals during treatments. Frie and her colleagues in the Michigan State University BLV research team hope that additional recommendations will continue to emerge as BLV research progresses. Their aim is that new research will help inform new policies and treatments that reduce the transmission of BLV. 'That is the real, ultimate goal of all of this research,' Frie says. 'To be able to contribute to a positive change in the industry that leads to reduced BLV infections and, hopefully, happier, healthier and more productive dairy cows.'

Meet the researcher

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Meredith Frie began her education in health and immune function at the University of California, San Diego, graduating with a Bachelor of Science in Human Biology in 2012. She went on to join the Cell and Molecular Biology PhD program at Michigan State University (MSU) after receiving a recruiting fellowship from the College of Natural Science. Frie has conducted her graduate research in the laboratory of Dr Paul Coussens, professor of Animal Science and Microbiology and Molecular Genetics. While at MSU, Frie has been the recipient of numerous research awards, including two American Association of Immunologists Trainee Abstract Awards, two American Association of Veterinary Immunologists Graduate Student Awards, and a USDA NIFA Pre-Doctoral Fellowship intended to enhance her important work on the immune-suppressing symptoms of Bovine Leukemia Virus infection on US dairy cattle. She expects to defend her doctoral dissertation on the health impacts of the virus in November 2017, with three peer reviewed publications to date. She hopes that her work with BLV will help shape US cattle management practices to eradicate the virus and improve the health, wellbeing and productivity of dairy cows.

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Michigan Milk Producers Association

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FIGHTING ENDEMIC PARASITES: NEW TECHNOLOGIES TO SOLVE OLD CHALLENGES

Intestinal worms are among the most common types of parasitic infections worldwide. Despite technological advances in other areas of medical diagnostics, the procedure for identifying worm infection, the faecal egg count, has remained largely unchanged since its debut nearly a century ago. **Dr Paul Slusarewicz** and the team at MEP Equine Solutions are revolutionising the way veterinarians detect and quantify worm infections using a tool many of us already carry on a daily basis – our smartphone.

A Worldwide Problem

Intestinal parasites are a major concern for human health in developing countries and veterinary medicine worldwide. Helminth worms, such as roundworms, are the most common type of intestinal parasite in mammals and pose a major health risk to infected host individuals. They feed off of the host and commonly cause symptoms such as poor nutrient absorption, anaemia, muscular weakness, impaired immune function, and in severe cases, intestinal blockage.

Preventing and treating intestinal worm infections is of paramount concern in many species, particularly in grazing animals such as horses. The life cycle of many intestinal parasites involves the deposition of eggs in manure in the hope of transmitting to a new host animal through accidental ingestion or direct contact with the skin. When one member of a herd of grazing animals is infected with parasitic worms, others easily pick up the infection by ingesting grass contaminated with eggs and larva.

Historically, the standard for diagnosing parasitic worm infection has been the 'faecal egg count' – by analysing stool samples under a microscope. While this procedure is fairly simple in theory (owners just collect faecal matter, and send it to a laboratory), it carries many limitations. First of all, microscopic visualisation of parasitic eggs typically cannot be done on site, it requires technical expertise and expensive microscopic equipment, and it is time consuming, often taking days to obtain results. The results are often inconsistent, as they rely upon the skill, judgement and level of fatigue of the person doing the counting. Also, two egg counts of the same sample are seldom the same, due to the variation inherent in the sampling procedure itself.

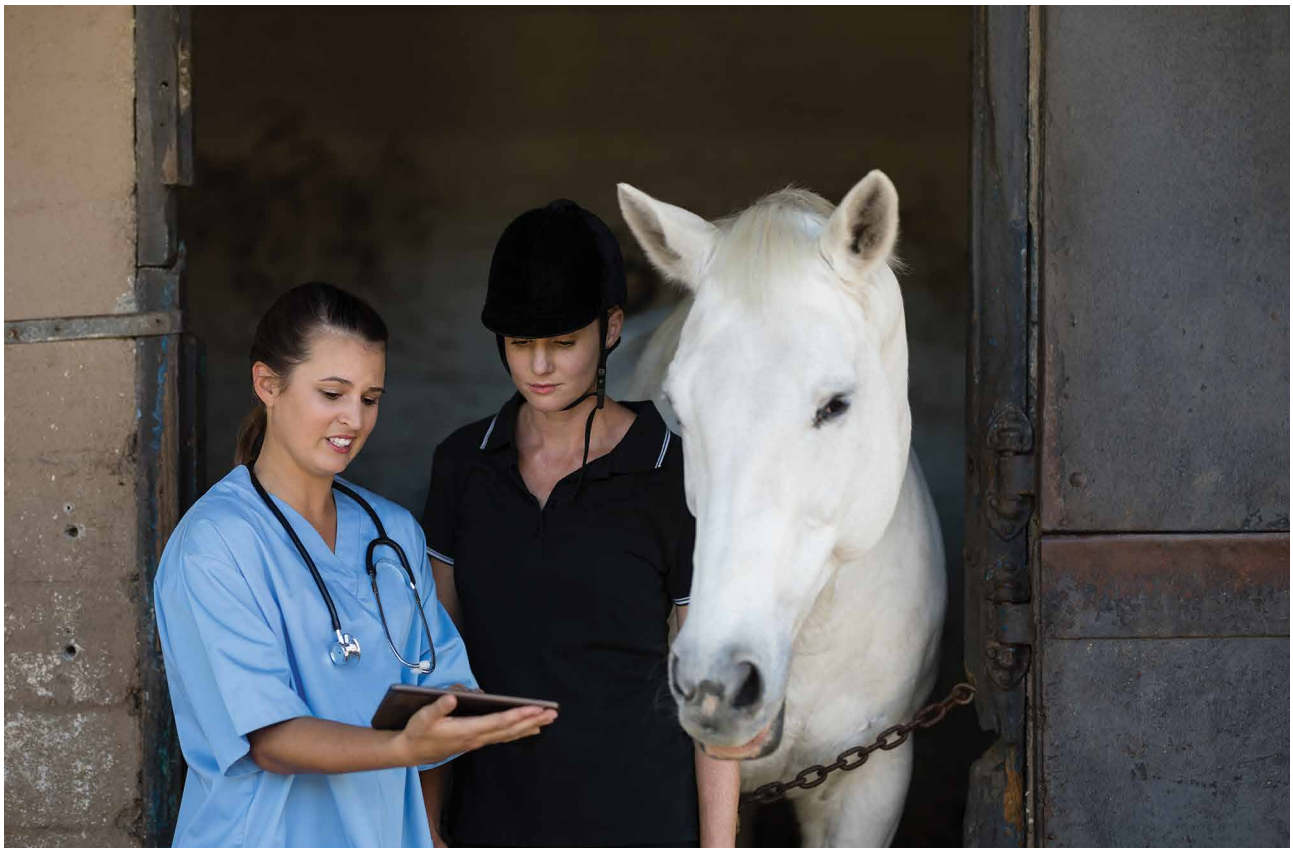
Because of these limitations, many horse owners choose to skip this test. Instead they give their animals deworming drugs prophylactically on regular schedules with no knowledge of the horse's infection status, even though only animals shedding high amounts of eggs need to be treated to keep levels of infection low in the herd. However, this strategy carries its own major limitation – since deworming treatments are given to all the animals and are not targeted to the highest shedders, many more parasites are exposed to these drugs that would be the case with targeted treatment. As a result, the most drug-resistant parasitic worms persist, and highly drug-resistant parasites are emerging at alarming rates.

Dr Slusarewicz and his partners recognised the need for a simple and fast stall-side method of determining parasitic worm infection in horses, empowering horse owners to offer targeted treatment options. Their company, MEP Equine Solutions, has developed a now patented procedure, which has the potential to transform how veterinarians and doctors approach the identification and treatment of parasitic worm infections.

Fresh Ideas in Diagnostics

Dr Slusarewicz began his career as a biochemist, working to solve common health problems at the molecular level. After spending the first half of his career studying human health, he and his CEO Eric Hauck realised that a technology they were working on together to repair tendon proteins could be readily translated to fill a need in equine medicine. The vision for improved parasite diagnosis began in

‘We envision a more sensible and precise method for faecal egg counting that is more convenient for veterinarians and animal owners. With this method, a microscope will no longer be needed, and the identification of eggs will not depend on the subjectivity of the person performing the test. This will all make it easy for everyone to do the right thing, by testing instead of treating prophylactically, and so prevent the ever-growing problem of parasite drug resistance.’



early 2014, when Hauck was introduced to Dr Martin K. Nielsen, a professor and renowned equine parasitologist at the Gluck Equine Research Center at the University of Kentucky. It was then that he came to realise the unmet need for improved diagnostics in clinical parasitology. The two lamented that although the faecal egg count was essential to modern veterinary medicine, the procedure had remained largely unchanged since its origin in the 1930s. The pair brought the problem to the attention to Dr Slusarewicz, whose experience in protein chemistry and analytical assay development was essential for addressing the problem.

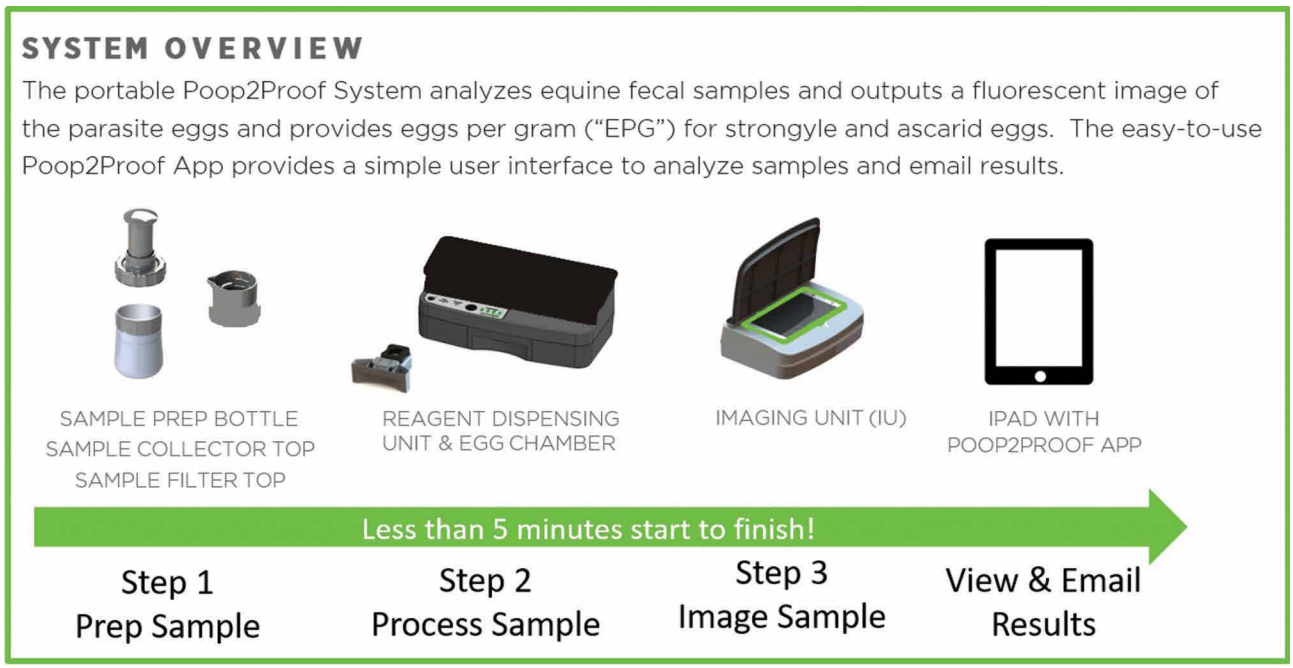
The trio founded MEP Equine Solutions and began research in June 2014 to tackle the challenge of modernising the faecal egg count. ‘We envision a more sensible and precise method for faecal egg counting that is more convenient for veterinarians and animal owners,’ says Dr Slusarewicz. ‘With this method, a microscope will no longer be needed, and the identification of eggs will not depend on the subjectivity of the person performing the test. This will all make it easy for everyone to do the right thing, by testing instead of treating prophylactically, and so prevent the ever-growing problem of parasite drug resistance.’

A Universal Marker for Parasite Eggs

Dr Slusarewicz realised that in order to develop an effective stall-side test for parasitic worms, he would need to find a better way of detecting helminth worm eggs in faecal matter. Calling on his training as a biochemist, he realised that the most efficient way to accomplish this goal would be to identify a compound that was ideally unique to egg casings and shared between multiple worm families – a universal egg marker.

Faecal matter often contains plant matter in various stages of digestion, along with mammalian cells from the host’s intestinal tract, and it would be critical to use a compound that would easily distinguish these components from the worm eggs. He settled on a carbohydrate called chitin as a key candidate for a universal egg marker that could be used in diagnostic procedures. Chitin is not found in either plant or mammal cells, but is a near universal component of insect exoskeletons, fungi and egg casings. With this feature in mind, Dr Slusarewicz and his colleagues chose chitin towards developing a next generation faecal egg counting protocol.

The team began by developing a biochemical tag, by attaching fluorescent dye to a protein that binds to chitin. Once they confirmed that the dye would attach itself to chitin molecules, they needed to confirm how effective the dye would be under the intended usage conditions. To do so, they went to the University of Kentucky Maine Chance Research Farm and local animal shelters to obtain faecal samples from horses, cattle, goats, sheep, dogs and cats. The team split each sample, with one half



used for a standard faecal egg count and the other half reserved to be tested with the team's new protocol. They then stained the faecal matter with the dye and viewed it under a fluorescent microscope. To their delight, the dye illuminated eggs from multiple species of helminth worm, making visualisation and counting much easier than with the classical method.

Harnessing the Technology in Your Pocket

The next phase the process was to make the counting process easier. The traditional method involves a laboratory technician performing the count, and requires an expensive microscope, a technician with specialised training, and a significant amount of time. Dr Slusarewicz and his colleagues wanted to investigate if the counting could be automated, and further, if it could be done using less expensive and more common equipment – such as a smartphone.

Dr Slusarewicz and a team of engineers built a specialised cradle that a smartphone can be placed into, to create consistent high-quality images of the dye-stained eggs. The cradle incorporates a high-magnification lens attachment and a light source to make the eggs glow, which focuses onto a grid where treated faecal samples can be loaded. With this setup, it became relatively easy to capture high-quality images of the fluorescent eggs, which can then be used in counts.

To reduce the time and technical expertise needed for a count, Dr Slusarewicz's next goal was to develop an image-processing program that could perform the egg counts automatically. To ensure that the program would not accidentally count other potential chitin-containing materials, such as fungal spores or fragments of injected insects, the team selected parameters designed to single out worm eggs.

Initially, these automated counts were performed by importing images onto a computer, but the team took the process a step further and developed a smartphone app – with the ability to do the same counts without transferring the image to a new device. With the combination of the photo cradle and app, the team was now able to use a single smartphone to image and analyse a sample.

To account for the various generations of smartphone technology in circulation, the team tested the setup with multiple phones of varying photographic strength. The results were astounding – even with the lowest resolution mobile camera, the rig was able to provide faecal egg counts consistent with and often more accurate than counts performed by a trained technician. Further, the app was able to distinguish between two different types of common and most pathogenic equine helminth worm eggs – strongyle eggs and ascarid eggs.

A New Vision for Parasite Detection and Treatment

The work of Dr Slusarewicz, the team at MEP Equine Solutions and the University of Kentucky, could prove to be transformative to the way helminth worm parasite infections are diagnosed and treated worldwide. A procedure that once took days to produce results can now be performed with startling accuracy in under 5 minutes out in the field by any veterinarian with a smartphone, which sends an email with the photo and record of the results to both the veterinarian and animal owner. Rather than applying monthly rotations of de-wormers to an entire barn full of horses and hoping for the best, horse owners can now provide targeted treatment that fully addresses the unique needs of a specific animal. Treatments may easily be tailored to a particular horse's parasitic load, shedding status, and the types of worms the animal is infected with. Horses will be spared unnecessary drug administration, and strategic application of de-wormers in infected animals will reduce the rates of drug resistance in helminth parasites.

The MEP team plans to expand the system's reach beyond the needs of the equine community, to revolutionise parasite diagnostics across multiple species and medical needs, possibly even to humans. Their next research directions involve refining the accuracy of their technology to identify more types of helminth worm eggs and produce a more precise laboratory version that runs on a desktop computer. The team has made amazing strides in parasite diagnostics over the past three years, and envision even greater advances in the years to come.

Meet the researcher

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Paul Slusarewicz began his research career at the University of London, where he obtained both his BSc and PhD in Biochemistry. His thesis and his postdoctoral work focused on the identification and purification of various proteins involved in transporting material around a cell. From there, he transitioned into industrial biotechnology and pharmaceutical R&D, quickly developing from a research scientist into an experienced leader, gaining expertise in product development and marketing. After successfully holding numerous high-level positions in various biotech firms, in 2014 Dr Slusarewicz co-founded his own start-up, MEP Equine Solutions LLC, in order to commercialise his invention of a novel quantitative assay for parasite egg load in faecal matter, where he currently serves as the Chief Scientific Officer. In addition to helping run his company, Dr Slusarewicz is an Adjunct Associate Professor in the Gluck Equine Research Center at the University of Kentucky.

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IN PURSUIT OF WILD GAME: INVESTIGATING PEOPLE'S PERCEPTIONS OF HUNTING

Hunting is among the most ancient of human activities, and still plays a major role in obtaining food for many people worldwide. **Dr Shawn Riley** and **Dr Göran Ericsson** work to understand the volume and distribution of wild-harvested meat, how this meat moves through society, and the effects of sharing the harvest on people's perspective toward traditional uses of wildlife such as hunting. They also investigate the factors that influence people's perception of hunting, and how hunters distribute their yields in different situations.

Recreational hunting provides funding for wildlife management that contributes to conservation, and is one of the primary methods for controlling populations of large game animals. Meat obtained from hunting can also form an important component of people's diets, reducing the reliance on intensive farming practices.

In many areas, there is a great deal of legislation tied to how and when hunting occurs, to promote sustainable populations of wildlife and protect people's safety in surrounding communities. If support for hunting is to be maintained, it must be relevant and acceptable to society. If support declines, this might present a major challenge to how wildlife is managed in many parts of the world. Thus, public support for hunting is key to preserving this traditional activity, which contributes to population control, culturally significant food supplies, and is a catalyst for social interactions between hunters and non-hunters.

As more and more people move into urban areas, political influence is increasingly concentrated in communities that have less of a direct connection to using natural resources in their daily lives. However, this political power shapes laws that affect the usage of natural resources in the rural areas surrounding their communities. For traditional uses of natural resources to persist, support and acceptance from people in urban populations will be necessary.

Given these shifts in the human population, a greater understanding of factors that influence the public perception of hunting is needed, taking into account the dynamics between urban and rural populations regarding hunting perceptions.

To address this, a productive US-European collaboration is developing between Dr Shawn Riley of Michigan State University and Dr Göran Ericsson of the Swedish University of Agricultural Sciences. This partnership started when Dr Ericsson hosted Dr Riley, who was on a Senior Fulbright Fellowship during a sabbatical leave. Dr Ericsson, himself a postdoctoral Fulbright Fellow in the 1990s at the University of Wisconsin, was no stranger to international collaboration. His graduate student, Per Ljung, then spent a half-year at Michigan State University, while Dr Riley's doctoral student, Amber Goguen, spent nearly a year in Sweden, working with Dr Ericsson and making vital contributions to the collaboration.

Although wild-harvested meat has been a central topic of Dr Riley and Dr Ericsson's shared research, the pair also investigates public perceptions of large predators, moose and wild boar. These sorts of relationships are invaluable to developing a shared understanding of the influence various forms of culture and governance have on the natural world,' says Dr Riley.

CREDIT: Shawn Riley

Perceptions of Hunting in Non-Hunters

There are many factors that potentially influence a person's perception of hunting. Previous research determined that social relationships with hunters and personal experience with hunting are both strongly associated with an acceptance of hunting practices. Many socioeconomic factors are associated with views of hunting – men, older generations, less educated populations, and those from rural backgrounds are more likely to take a favourable stance towards hunting. In Sweden, urbanites with parents who also grew up in urban areas are less likely to favour

hunting, while those who grew up in rural areas or had parents who did are more likely to accept hunting. It has also been found that people are far more accepting of hunting that is done with the purpose of obtaining meat, rather than hunting solely for sport or to obtain trophies.

In Sweden, as in most of Europe, it is legal to sell wild-harvested meat through commercial channels so that it can be obtained in grocery stores, though it is predicted that most wild meat is obtained through trade and barter within social networks, similar to those found in the United States. Approximately 2% of meat consumption in Sweden is from wildlife, and many stores sell both farm-raised and wild-harvested meat. Drs Riley and Ericsson and their colleagues wondered if exposure to wild-harvested meat in urban populations influenced people's perceptions of hunting.

The team predicted that urban individuals who consume wild-harvested meat would be more likely to harbour positive perceptions of hunting than those who did not eat wild-harvested meat. They sent questionnaires to 1067 randomly selected Swedish residents, aged between 16 and 65, with questions related to hunting and consumption of wild-harvested meat. Selected individuals were sent a pre-notification a few days prior to the full survey, followed by a thank you note.

The team's survey asked about the individual's personal experience with hunting, family members and friends that hunt, how often they ate wild-harvested meat, whether or not they felt hunting was cruel to animals, and if they were ever hindered from doing activities in the forest during hunting season. The team received 488 useable responses, but since they wanted to hone in on non-hunters, they excluded responses from 31 participants who indicated that they had hunted in the past year.

The final set of 457 non-hunter respondents were evenly split between men and women, and included mostly current city dwellers, about half of which had spent their entire lives in a city. They found that wild-harvested meat was consumed at least once a year in 65% of non-hunter households, and this consumption was strongly associated with positive views of hunting. Of the non-hunter respondents, 80% held favourable views of hunting, with the biggest factors in favourable views being social relationships with hunters and consumption of wild game meat. Dr Riley and Dr Ericsson suggest that a broader distribution of wild-harvested meat could help to improve people's views of hunters and hunting.

Differing Perceptions

The research team's next goal was to tease out the distinctions between rural and urban non-hunters. Ongoing high levels of urbanisation and the political influence of urban populations on rural communities mean it is more critical than ever to shape perspectives of hunting if rural communities hope to hang on to their cultural traditions and continue aiding in controlling wildlife populations. As highlighted by the team's survey, two of the most important factors that influence a person's perception of hunting are their personal relationships with hunters and whether or not they eat wild-harvested meat. The researchers predicted that urban non-hunters would harbour more negative views of hunting than their rural counterparts.

To probe the question of whether rural non-hunters view hunting more positively than urban non-hunters, Dr Riley and Dr Ericsson focused their attention on the north-south orientation of Sweden. Northern Sweden is largely rural, while southern Stockholm is highly urban. They randomly selected 150 residents of each of the 26 counties in the Stockholm region, and in each of the 69 counties of the rural northern regions, to send hunting perception surveys to. In total, 3,900 urban individuals and 10,350 rural individuals received surveys. The contact

CREDIT: Amber Goguen

strategy involved an initial pre-notification of survey selection, followed by the full questionnaire, and then a thank you note.

This survey included questions about the respondents' current community, such as the population size of their town, along with demographic questions. They were asked if they hunted, whether or not their parents had hunted, if they had any friends that hunted, and how often they consumed wild-harvested meat. Finally, respondents were asked to rate their attitude towards hunting, along the scale of positive, accepting, hesitant, and negative. They were also asked how they felt about hunting large animals, such as moose and deer, for recreation versus for meat.

From this second Swedish survey, Drs Riley and Ericsson received 1,596 urban non-hunter responses and 4,211 rural non-hunter responses. Many rural non-hunters reported having friends (82%), household members (26%) or parents (34%) who hunted, while rates were much lower among the friends (52%), household members (3%), and parents (11%) of urban respondents. Trends for wild-harvested meat consumption were similar, with 81% of rural respondents eating wild game at least once a year or more, while 62% of urban respondents reported the same. Attitudes also reflected these trends, as 70% of the rural northern non-hunting Swedes reported favourable views of hunting, while only 48% of urban non-hunters did. Among all groups, there was considerably greater support for hunting for meat than hunting for sport. Reflecting the team's earlier reported trends, those who knew hunters personally or grew up with hunting parents were much more likely to view hunting favourably, as were respondents that regularly ate wild-harvested meat.

Once again, these results support the idea that linking hunting to positive outcomes, such as social relationships or tangible resources like meat, can help fuel positive perceptions of traditional uses of wildlife such as hunting. The effects of sharing and consuming wild-harvested meat were practically the same in rural and urban settings – wild-harvested meat simply was more available in rural locations than cities.

Programs or incentives that help hunters broaden the distribution of wild-harvested meat, whether in urban grocery stores or through broadened social networks, could help improve public perception of hunting as a means to obtain food. A simple gesture that every hunter could use to improve the relevancy of hunting is invite someone to dinner!

Perceptions of Hunting in the United States

In the US, the sale of wild-harvested meat is prohibited, but people may legally obtain such meat through social and trade relationships with hunters. This is different from European countries, where people may easily obtain wild meat without personally knowing a hunter. Although commercial distribution of wild-harvested meat can easily be tracked in Europe, understanding the networks through which such meat is traded in the US is more complex.

'We are aiming to better understand the dynamics in the US and how they compare with Europe, where wild-harvested meat typically can be sold and purchased in a formal market system,' says Dr Riley. He and Dr Ericsson are working with wildlife managers and researchers to describe the yield, use and distribution of meat obtained by recreational hunting. In the state of Michigan, many hunters complete a yearly questionnaire – the Michigan Deer Harvest Survey – at the end of each hunting season. The team worked with the Michigan Department of Natural Resources to include additional questions about wild meat use and sharing in the 2013 mailing of the survey. Standard questions included the total number of deer and hunting equipment used, while the new questions asked hunters if they share their venison, who they shared it with (family, friends, etc.), and asked them to estimate the total number of people they gave meat to.

The researchers received 19,981 useable responses to the survey, and were able to estimate that 11,402 to 14,473 metric tons of wild venison had been harvested that year. At least 85% of hunters shared their yields with other people, with sharing most common with household members (68%), extended family (52%), and friends, neighbours, and colleagues (50%). The average hunter shared their wild-harvested venison with 5.6 people, creating a vast distribution network.

Drs Riley and Ericsson estimate that approximately 1.9 million people, or 19% of Michigan's population, received wild-harvested venison in 2013, despite only 6% of the state's population engaging in hunting. In the absence of a formal market for sharing wild-harvested meat, hunters were still achieving a wide distribution through their social networks. These networks of hunters serve to not only provide food for a wide range of people, but to provide ecosystem services for rural areas by controlling deer populations and reducing their effects on ecosystems. The networks, as a rule, are typically tight. Hunters have the opportunity through their sharing behaviours to expand the distribution of this culturally important food source and increase the relevancy of hunting. The team estimates that these services could easily represent 1.5 billion dollars per year in value for the US. Dr Riley points out, however, that 'there is much more to the act of sharing of wild-harvested meat than the utilitarian value of meat. The vast majority of hunters in Sweden and the US share their harvest, which signals the cultural importance of the act of sharing as well as the meat. The stories, the connections with nature, and the conversations over a shared meal are part of what it means to be human.'

Understanding Hunting Dynamics

The team's work is helping natural resource and wildlife management organisations understand how recreational hunting fits in to the local ecosystem of human and wildlife interactions. As urbanisation increases, this knowledge will grow ever more critical in shaping legislative decisions that could impact hunting communities and practices around the world. The research being achieved through the international collaboration between Dr Riley and Dr Ericsson serves as a step toward greater understanding of the relationship between humans and nature, and the influence of different systems of governance.

Meet the researchers

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Dr Shawn Riley began his formal education at Montana State University, where he earned a BS in Biological Sciences, and a MSc in Fish and Wildlife Management. He worked as a practicing wildlife biologist in Montana for 13 years before taking a mid-career break to earn a PhD in from Cornell University with a focus on environmental economics and social psychology. He founded his current laboratory at Michigan State University in 2001, where he currently serves as Parrish Storrs Lovejoy Professor of Wildlife Management in the Department of Fisheries & Wildlife. His current research includes causal factors in the decline of hunting; factors affecting acceptance of wildlife in suburban settings; social dynamics of sharing and receiving wild-harvested meat in US and Sweden; social perceptions of large carnivores in Sweden; and, factors affecting successful partnerships with state wildlife agencies.

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Dr Göran Ericsson started his research career at Uppsala University, earning an MSc in Biology. He continued on to the Swedish University of Agricultural Sciences to earn his PhD in Animal Ecology, forming his research group at the same university in 2002 where he currently serves as a fully tenured Professor in Wildlife Ecology and the Department Chair of Wildlife, Fish, and Environmental Studies. He has supervised nearly 50 graduate students in wildlife ecology, and developed advanced curriculum for undergraduate students called 'Human Dimensions of Fish and Wildlife'. He maintains an active hand in research related to moose, interspecific competition, artic issues, climate change, and human dimensions of living with wildlife.

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SURROGATE BACTERIA FOR FOOD SAFETY

In the wake of a terrible *E. coli* food poisoning outbreak, a group of food scientists decided to gear their research towards improving the safety of meat and poultry, whilst reaching out to food industry stakeholders and forming a collaboration known as the **Consortium of Food Process Validation Experts (CFPVE).** As part of this important collaboration, CFPVE scientists are advocating the use of non-pathogenic bacterial substitutes for process validation in processing plant environments.

One of the most infamous cases of food poisoning in modern times was an outbreak of *E. coli* in undercooked hamburgers served at Jack-in-the-Box restaurants in 1993. The undercooked beef patties were sold in 73 Jack-in-the-Box restaurants, and 732 people were infected, the majority of whom were under 10 years old. Tragically, four children died and 178 other victims were left with permanent injuries, including brain and kidney damage. It was determined that high demand for a promotional sandwich had overwhelmed local restaurants, which were required by Washington state law to cook burgers to 155°F (68°C), an end-point temperature that would have probably prevented the outbreak, but instead adhered to an outdated federal standard of 140°F (60°C). Moreover, an investigation identified six slaughterhouses in the US and Canada as the 'likely sources of the contaminated lots of meat'. In February 1998, Jack-in-the-Box's holding company, Foodmaker, agreed to accept $58.5 million from Vons and eight other beef suppliers to settle the lawsuit filed in 1993.

While this was by no means the first such case, this was different because it had exposed deep issues in the meat supply chain that needed to be resolved. It was clear that food safety required a higher priority. While some argued that the responses of the industry and regulators were 'too little, too late', the tragedy did cause the beef industry to shift its focus from shelf-life to safety.

In the wake of this outbreak, food microbiologists from various US institutions – including Texas A&M, Iowa State, Pennsylvania State, Texas Tech, Oklahoma State, Kansas State, Colorado State, University of Georgia, University of Arkansas, University of Wisconsin-Madison and the USDA-ARS – geared their research interests toward developing pathogen interventions that could be applied in food processing. A need for proving effectiveness of implemented interventions led these scientists to promote a more robust and scientific approach to validation of meat manufacturing processes. Their efforts eventually led to the creation of the Consortium of Food Process Validation Experts (CFPVE).

With the introduction of the new Preventive Controls for Human food (FSMA-PCHF), there is an increasing need for assistance amongst processors. Similarly, consumption of meat in the US and across the developed world is rapidly increasing, and the need for robust food safety validation has never been greater. There are about 9.4 million cases of illnesses caused by foodborne pathogens in the US every year, with the leading causes being norovirus (roughly half of the illnesses), and bacterial pathogens such as *Salmonella*, Shiga toxin-producing *E. coli* (STEC), *Clostridium perfringens*. *Listeria monocytogenes* and *Campylobacter* contributing to the total.

The pathogenic bacterium behind the infamous Jack-in-the-Box outbreak was the deadly *E. coli* O157:H7 strain, which is a major cause of food-borne illness worldwide. Infection with O157:H7 can lead to bloody diarrhoea and even kidney failure, and young children and the elderly are particularly susceptible. In 1994, in the wake of the Jack-in-the-Box outbreak, the US Department of Agriculture, Food Safety and Inspection Service (FSIS) banned the sale of ground beef contaminated with the strain. In the US, in addition to the O157:H7 serotype of *E. coli*, there are six additional regulated serotypes of STEC banned in meat.

STEC cells are naturally found in the intestinal tracts of cattle, sheep and goats. The risk of beef contamination can be reduced by applying proper antimicrobial interventions during the slaughter and processing of the carcass – interventions such as hot water washes, acid sprays and applying food grade antimicrobials. After the Jack-in-the-Box outbreak, the collaboration of food scientists tested various carcass and sub-primal cut interventions in the laboratory and pilot plant facilities, including hot water sprays, acetic acid, lactic acid, and ozone treatments.

These experiments were helpful in identifying effective bacterial interventions – but slaughterhouses where cattle are converted into cuts of meat under real-world conditions can be very different than the team's laboratories and pilot plants. They then attempted to test the interventions in actual slaughter plant facilities under real-world conditions, but soon ran into a few snags. In actuality, O157 is extremely difficult to find. When present, it is likely to be located on the carcass surface, with very low numbers of cells constituting an infectious dose. And to

make things even more complicated, the contamination is not evenly distributed. That made it very difficult to validate the effectiveness of the team's laboratory-tested interventions in real life. In the lab, the team had intentionally contaminated carcass surfaces with O157, and measured the reduction in bacterial populations after each intervention. But this would be disastrous in a slaughterhouse, even for validation purposes.

HACCP validation really began to come of age in the food processing industries around 2012 when several regulatory documents were published providing guidance. HACCP is a systematic approach employed by food processing facilities to prevent biological, chemical and physical hazards that can compromise food safety. HACCP started as a collaboration between NASA, the Pillsbury Company and the US Army laboratories to provide safe food for space expeditions – and ended up being the standard quality assurance system for food on Earth!

At the heart of implementing a HACCP system and the new FSMA-PCHF is 'validation'. Process control within a food processing facility must be validated to ensure that controls are working to eliminate or control hazards. To demonstrate that a processing facility is capable of controlling a foodborne pathogen such as *Salmonella*, *Listeria monocytogenes* or STEC through various interventions, an ideal experiment would involve intentional contamination with the above-mentioned hazards followed by a demonstrated reduction of the bacterium through the process. However, introducing dangerous pathogens in a processing facility would be unwise and potentially hazardous. But how then could validation be carried out to assure interventions are functioning as designed to preserve food safety?

Surrogate Bacteria

It was at this time that two members of the CFPVE, Dr Gary Acuff and Dr Jim Dickson, came up with a solution. They began researching the possibility of using 'surrogate' bacteria in microbial validation studies – non-pathogenic bacterial strains that could be used to simulate the pathogen of interest. An ideal surrogate should have very similar biological characteristics to their pathogenic counterpart – and must show no pathogenicity whatsoever. Drs Acuff and Dickson spent several months collecting bacteria from cattle feedlots and swabbing the hides of cattle in their quest for an ideal surrogate to O157.

They later tested their collected bacteria in the lab, and compared their growth rates, heat resistance and acid resistance with those of *E. coli* O157. Basically, they were looking for a non-pathogenic twin for *E. coli* O157. They didn't find any single strain with exactly the same features as O157, but did isolate five strains that represented *E. coli* O157 fairly closely when used together in a mixed culture. The pair then tested this cocktail of five strains against various carcass interventions and found that they matched up well to what they had reported for *E. coli* O157. Hence, they tested them in actual slaughter facilities by contaminating carcass surfaces with the cocktail, and then measuring the numbers of bacteria before and after the carcass treatments. The team's results would be indicative of what would happen if the interventions were used against the target pathogen, *E. coli* O157. These cultures are now available through the American Type Culture Collection (ATCC).

The Consortium of Food Process Validation Experts

The ATCC is a non-profit organisation that acts as a national repository for microbial strains for microbiological standards, as well as cell lines for culturing – essentially an 'open access' microbial zoo that

researchers anywhere can use. The surrogate strains were donated to the ATCC collection so that anyone could use them, in the hopes that they would become a standard part of HACCP verification. 'As food microbiologists, we felt the process for validating a carcass intervention was very clear,' states Dr Acuff. However, the meat and poultry industry didn't initially share this view, and they received more industry resistance than they bargained for. For industry officials, the thought of contaminating some of their carcasses with bacteria, albeit harmless bacteria, was alarming – even for the purpose of safety validation!

It was painfully clear that there was a chasm between food microbiology laboratory research and food industry in-plant process validations, in both theory and practice. It was at this time that the group of collaborating food scientists decided to form a consortium to bridge this gap, bring diverse process and product expertise, and implement a more scientifically-informed approach to food safety validation. It was at the International Association for Food Protection (IAFP) annual meeting of 2011 that the scientists decided how this would be organised, and the CFPVE was born.

From the outset, the CFPVE has worked hard to support food industry stakeholders in promoting and applying scientifically sound approaches and protocols for food process validation, providing practical, standardised and unbiased interpretations of existing science, guidelines and policies, and controlling hazards inherent in food production, especially bacterial pathogens. A major part of the Consortium's work is extensive dialogue and outreach with stakeholders from industry, government and academia through a variety of channels – fact sheets, publications, white papers, training materials, web sites, videos, workshops, webinars and podcasts.

Furthermore, members of the Consortium regularly consult with small and medium scale food processors to assist them with validation and verification activities and to help them develop new 'Food Safety Plans'. Reviewing existing food safety plans for such processors is a critical activity that the members of the Consortium are involved with, as this helps determine the compliance of the processors to the new FDA regulations.

The Consortium's formation was timely, as the need for an unbiased, effective and unified approach to food safety has never been greater.

Food process validation is without a doubt essential, but there is currently a degree of uncertainty as to how validation should be used to assure food safety – as the industry's initial resistance to using surrogates attests. This is more than just about promoting surrogates in HACCP verification – the CFPVE is helping industry to verify HACCP 'critical control points' beyond microbiological testing of finished products. The expertise supplied by the CFPVE is helping processors assure safety of their food products through properly conducted validation of antimicrobial interventions and food manufacturing practices, especially meat and poultry, and as a result minimising the risk of pathogen-related food poisoning for consumers.

As well as being actively involved in the CFPVE, several members of the CFPVE have continued their quest for suitable surrogates as well as approaches and protocols for application to HACCP. This work has gone beyond *E. coli* O157 and all regulated STEC serotypes and has focused on testing interventions to reduce *Salmonella* contamination in meat, poultry and other foods. Significantly, the team has found that mixed cultures of non-pathogenic *E. coli* strains can also act as suitable surrogates for *Salmonella*. They have microbiologically tested meat, poultry and food processing interventions beyond slaughter, including washing beef carcasses with lactic acid and trisodium phosphate, refrigeration and frozen storage and fermenting meat for sausage production. These surrogates are being evaluated for their behaviour in other novel food processing processes such as high-pressure processing, radio frequency heating and others.

The team's introduction of surrogates in food safety validation emphasises the need for strong collaboration and alignment among the food industry, policy makers and academia to provide us with food that is high-quality, nutritious and safe. While the risk of foodborne illnesses cannot be eradicated completely, robust process validations and responsible practices by farmers, slaughterhouses, processors, distributers, retailers, restaurants – and indeed consumers – can minimise the risk significantly.

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the Consortium of Food Process Validation Experts

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Trees for Cities

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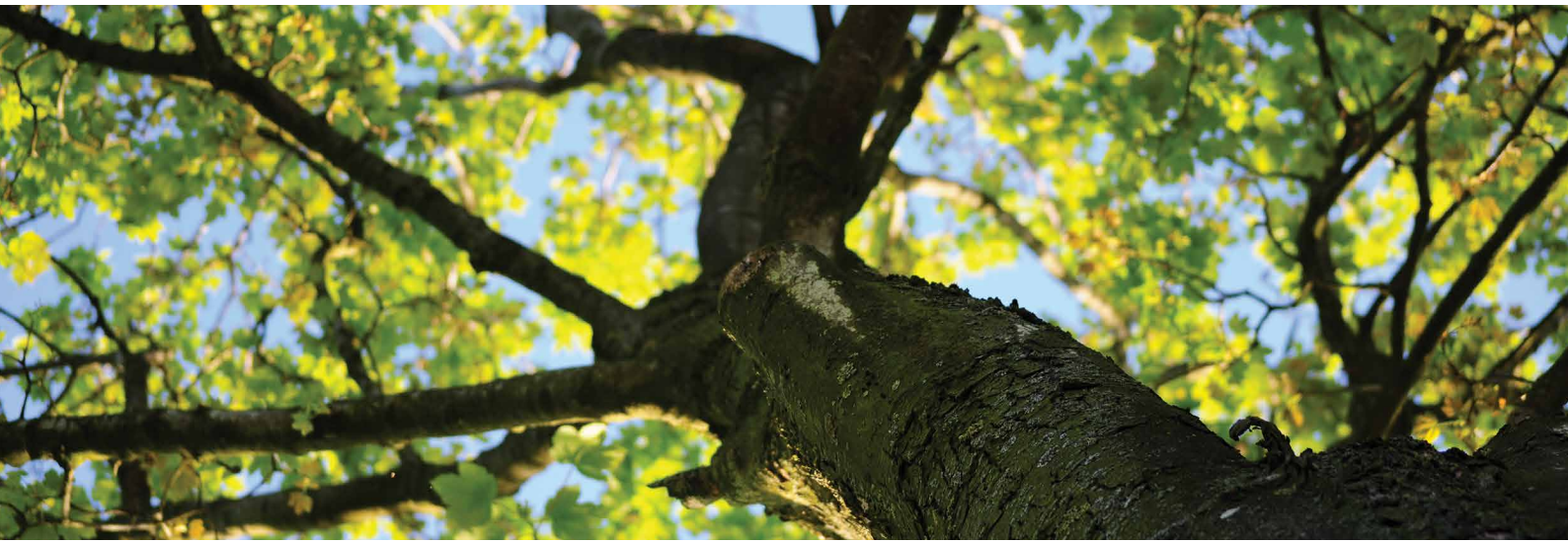

WHO WE ARE

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.

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