



# Scientia

FROM THE COSMOS TO LIFE  
ON EARTH

## EXCLUSIVES:

- The National Institute of Food and Agriculture
- The Laser Interferometer Gravitational-Wave Observatory

## HIGHLIGHTS:

- Investigating Plasma Storms and Substorms in our Near-Earth Backyard
- Predicting Climate Change Impacts: Regional Climate Modelling is a Critical Tool
- Marine Microbes Shed Light on our Changing Oceans
- Math Plus Biology: Building a Knowledge Base to Engineer Plant Traits



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## WELCOME...

It is my absolute pleasure to introduce this fascinating new edition of Scientia, where we explore the latest in earth, environmental and ecological research, with a pinch of cosmology thrown in for good measure.

To open the issue, we start big – with the universe. Here, we have had the pleasure of speaking with Dr Kiwamu Izumi, a scientist working at The Laser Interferometer Gravitational-Wave Observatory (LIGO). Dr Izumi tells us all about LIGO's ground-breaking gravitational waves discovery – reported earlier this year. Also headed for a breakthrough are the NewDark scientists at the Laboratory for Theoretical and High Energy Physics in Paris, who are busy hunting for dark matter – that elusive substance that comprises 84% of the matter in the universe. From here, we start to bring things closer to home, first by investigating the solar wind, and the spectacular auroral displays it generates upon clashing with our Earth's magnetosphere.

Next, we reveal the latest in earth and environmental science research. With global temperatures on the rise, and species becoming extinct at a rate of more than 1000 times the 'background extinction rate', dedicating our research efforts to our environment is now more important than ever before. Here, we highlight cutting edge climate science research, along with several innovative new ways to battle pollution and save our declining fish populations. From here, we explore our oceans – where we feature four remarkable research projects, ranging from creating 3D images of deep-sea hydrothermal vents to exploring how marine microbes are adapting to our changing seas.

In our next section of the edition, we explore the diversity of life on Earth, by showcasing no less than 10 fascinating projects investigating the ecology and evolution of microbes and animals. Not wanting to ignore the plant kingdom, our final section is dedicated to our leafy cousins. Here we highlight the latest in crop science, and also introduce Dr Sonny Ramaswamy, the director of the US National Institute of Food and Agriculture (NIFA), in an exclusive interview about NIFA's activities in working towards a future of sustainable agriculture.

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Published in the UK, by  
Science Diffusion Ltd

ISSN 2059-8971 (print)  
ISSN 2059-898X (online)

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# CONTENTS

ISSUE : #109

- 04** MASSIVE LEAPS FOR COSMOLOGY AND PLANETARY SCIENCE
- 06** THE LASER INTERFEROMETER GRAVITATIONAL-WAVE OBSERVATORY (LIGO)  
The gravitational waves discovery: an interview with Dr Kiwamu Izumi, scientist at LIGO
- 10** DARK IS THE NEW BLACK  
[The NewDark group](#)  
Hunting for elusive dark matter
- 14** INVESTIGATING PLASMA STORMS AND SUBSTORMS IN OUR NEAR-EARTH BACKYARD  
[Dr Tony Lui](#)  
Understanding the mechanisms of space plasma interactions with Earth's magnetosphere
- 18** USING RADIOISOTOPES IN VOLCANIC CRYSTALS TO MEASURE THE AGE OF THE EARTH  
[Professor Urs Schaltegger](#)  
Analysing isotopes of uranium and lead in zircon to date geological events
- 23** SAVING OUR DYING PLANET, WITH SCIENCE!
- 25** PREDICTING CLIMATE CHANGE IMPACTS: REGIONAL CLIMATE MODELLING IS A CRITICAL TOOL  
[Professor René Laprise](#)  
Improving our understanding of climate change through the use of Regional Climate Modelling
- 30** ANTHROPOGENIC CONTRIBUTIONS TO THE SAHEL MEGA-DROUGHTS  
[Professor Yongkang Xue](#)  
Using multi-model efforts to investigate the Sahel region's 30-year long drought
- 34** ONE, TWO, THREE, BREATHE  
[BreezoMeter](#)  
An innovative new technology to address the problems caused by air pollution

- 38** ERADICATING WATER POLLUTION ACROSS THE GLOBE – WITH TREES  
[Professor Theodore A. Endreny](#)  
Planting trees to make our waterways safe for swimming, fishing and drinking
- 42** SHRINKING FISH STOCKS: THE EFFECT OF ENVIRONMENTAL SEX HORMONES ON IMMUNITY  
[Professor Helmut Segner](#)  
Exploring why freshwater fish stocks are dwindling, by studying how oestrogens affect fish immunity
- 46** GROWING BETTER SALMON: BALANCING ECONOMICS WITH ENVIRONMENTAL IMPACT  
[Dr Daniel Heath, Dr Oliver Love, Dr Bryan Neff, Dr Dennis Higgs, Dr Christina Semeniuk, Dr Trevor Pitcher and Dr Brian Dixon](#)  
Making Chinook salmon aquaculture more sustainable and environmentally friendly
- 50** EXPLORING OUR OCEANS
- 52** INVESTIGATING WHAT GOES ON AT THE BOTTOM OF THE DEEP BLUE SEA  
[Dr James T. Potemra](#)  
Monitoring the bottom of the ocean with the ALOHA Cabled Observatory
- 56** THE LAST UNEXPLORED PLACES ON EARTH  
[Dr Karen Bemis, Dr Darrell Jackson and Dr Guangyu Xu](#)  
Exploring hydrothermal vents using the Cabled Observatory Vent Imaging Sonar (COVIS)
- 61** UNCOVERING THE MYSTERIES OF MARINE MERCURY  
[Professor Robert Mason](#)  
Studying how mercury levels in the oceans are changing due to climate change

- 65** MARINE MICROBES SHED LIGHT ON OUR CHANGING OCEANS  
[Professor Gordon Taylor](#)  
Investigating the decline of dissolved oxygen in the ocean due to climate change, and how this affects microbial life
- 69** THE EVOLUTION OF LIFE ON EARTH
- 71** DECIPHERING UNKNOWN AND UNRECOGNISED PHOSPHORUS-MICROBIAL TRANSFORMATIONS  
[Dr Diliانا D. Simeonova](#)  
Studying how bacteria use phosphorus to gain energy, survive and proliferate in extreme environments
- 75** UNSEEN WORLDS: MICROSCOPIC LAKE PLANKTON FUEL FOOD CHAINS AND BEFRIEND ALGAE  
[Dr Bettina Sonntag](#)  
Exploring the lives of ciliates – unicellular organisms that live in freshwater lakes
- 79** CONTAGIOUS CANCER CELLS IN SHELLFISH  
[Professor Stephen Goff](#)  
Investigating how shellfish can develop cancer
- 83** HOW ANIMALS FABRICATE BIOMINERALS  
[Professor Daniel John Jackson](#)  
Studying how animals evolved to fabricate hard parts, by investigating molluscs
- 87** BEYOND GENETICS  
[Dr Craig Albertson](#)  
Investigating the genetic and environmental factors behind the evolution of the skeleton
- 91** COLD AS ICE: ANTIFREEZE PROTEINS IN POLAR FISHES  
[Professor Arthur DeVries](#)  
Studying antifreeze proteins that have evolved in polar fishes, enabling them to survive in polar environments
- 95** EXPLORING EVOLUTION BY STUDYING BEETLES LIVING ON THE EDGE  
[Professor Nathan E. Rank and Professor Elizabeth P. Dahlhoff](#)  
Exploring evolutionary responses to climate change by studying the Sierra willow leaf beetle



- 99** SOCIAL BEHAVIOUR IN BIRDS: WHAT MAKES THEM TICK?  
[Professor Elizabeth Adkins-Regan](#)  
Investigating physiological mechanisms underlying social and reproductive behaviour in birds
- 103** FORGING A HEALTHY RELATIONSHIP WITH NATURE THROUGH THE MARRIAGE OF SCIENCE AND ETHICS  
[Professor John Vucetich](#)  
Studying wolves and moose in Isle Royale and Yellowstone National Parks
- 107** BREAKING FREE THE STONES OF THE PAST  
[Professor William Hunt and Professor Ralph Hartley](#)  
Investigating the mysterious cairns in the coastal mountains of Alaska
- 111** FEEDING THE GLOBAL POPULATION
- 112** NATIONAL INSTITUTE OF FOOD AND AGRICULTURE (NIFA)  
An exclusive interview with Dr Sonny Ramaswamy, the director of NIFA
- 117** IMPROVING CROPS BY GENETIC ENGINEERING AND TARGETED GENOME EDITING  
[Professor Joyce Van Eck](#)  
Improving the ability of tomato and potato crops to resist disease using CRISPR/Cas9
- 121** THE SEEDS WE SOW AND THE GRAIN WE REAP  
[Dr Corina Vlot-Schuster](#)  
Studying the natural plant defence system known as Systemic Acquired Resistance, or SAR
- 125** MATH PLUS BIOLOGY: BUILDING A KNOWLEDGE BASE TO ENGINEER PLANT TRAITS  
[Professor Daniel Szymanski](#)  
Investigating the mechanisms underlying leaf shape, towards designing better crops





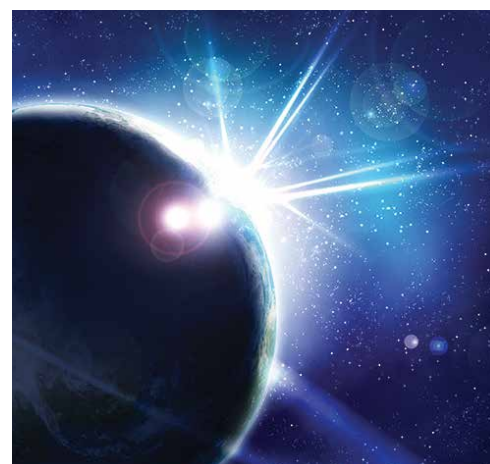
## MASSIVE LEAPS FOR COSMOLOGY AND PLANETARY SCIENCE

To open this edition of Scientia, we begin with the universe! That vast, unfathomably enormous expanse, which – as far as we know – encompasses the entirety of existence.

There has never been a more exciting time for space research. Just last year, gravitational-waves were observed by scientists at the Laser Interferometer Gravitational-Wave Observatory (LIGO) for the very first time. These ripples in the fabric of space time are generated by accelerating massive bodies – such as the pair merging black holes behind the observations made at LIGO. This discovery was of huge significance, as it marked the last remaining test of Einstein's theory of general relativity – a model of the universe where gravity arises due to the curvature of spacetime.

In this section of the edition, we have had the pleasure of speaking to Dr Kiwamu Izumi, a researcher working at the LIGO Hanford detector site. In this exclusive interview, Dr Izumi tells us all there is to know about gravitational waves (well, not quite!) and what the future holds for this new field of gravitational wave astronomy.

From here, we move on to a cosmological phenomenon that has not yet been directly detected – dark matter. Remarkably, this unidentified form of matter is estimated to comprise 84% of all the matter in the observable universe, and is not expected to be made up of normal atoms. Even though we can't see it, we know that dark matter is there, due to its gravitational interactions. Some of the first strong evidence for this elusive substance came with the findings of the American astronomer, Vera Rubin, who accurately measured the speed of stars in rotating galaxies. While Kepler's law predicts that in spinning galaxies, a star further from the galactic centre should be rotating around it much slower than a closer one, Vera observed that all stars were rotating at roughly the same velocity, regardless of their distance from the centre. This observation can be explained if the stars are not actually rotating around the supermassive black hole at the centre of the galaxy, but around many other unknown centres that provide gravitational attraction. Because we can't see this matter, attempts to explain its presence gave rise to the field of dark matter research.



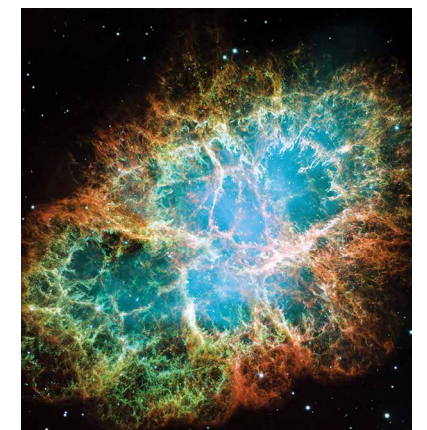
And this is not the only evidence we have for the presence of dark matter. Since it possesses mass, it can bend spacetime – as described by Einstein's hundred-year-old theory of general relativity, mentioned above. This leads to a phenomenon known as gravitational lensing – where light passing through a region of dark matter density becomes warped – something we can observe using telescopes. Last but not least, is evidence offered by the cosmic microwave background – light emitted in the early universe just 380,000 years after the big bang, which has been red-shifted to such an extent that we now observe it as low-energy microwave radiation. Unexplained fluctuations (anisotropies) in this background radiation are said to be due to the presence of dark matter in the early universe.

In the second article of this section, we are thrilled to introduce the NewDark team at the Laboratory for Theoretical and High Energy Physics at Sorbonne University in Paris. This collaboration brings together expertise from the multiple overlapping fields involved in dark matter research, to ultimately uncover the true nature of this mysterious substance.

Next, we start to bring things a little closer

to home, by investigating solar storms, and examining how they affect our home planet. Here, we showcase the work of space physicist Dr Tony Lui at The Johns Hopkins University Applied Physics Laboratory, who has the enviable job of studying aurorae – magnificent light displays caused by the interaction of the solar wind with the Earth's magnetosphere. Dr Lui has spent four decades unveiling the complex interactions between Earth's magnetic field and this plasma ejected from the Sun. Gaining a better understanding of these physical processes that lead to near-Earth plasma disturbances will allow us to forecast these phenomena that disrupt our satellites, communications systems and electronics.

For the last article in this section, we jump from the Earth's upper atmosphere to land on solid ground. Here, we introduce geophysicist Professor Urs Schaltegger and his team at the University of Geneva in Switzerland, who determine the time frame of geologic events such as mass extinctions. Because radioactive uranium decays into lead over time, the team are able to calculate the age of geological deposits by measuring the levels of uranium and lead isotopes found in zircon crystals. In particular, Professor Schaltegger and his team use this



'geochronological' technique to investigate the Permian-Triassic extinction (252 million years ago), the Triassic-Jurassic extinction (201 million years ago, which resulted in the extinction of about half of all species, allowing the dinosaurs to rise to prominence), and the Pliensbachian-Toarcian extinction (183 million years ago). From their results, the team have been able to conclude that volcanic activity was behind much of the massive shifts in life on Earth.





## THE LASER INTERFEROMETER GRAVITATIONAL-WAVE OBSERVATORY

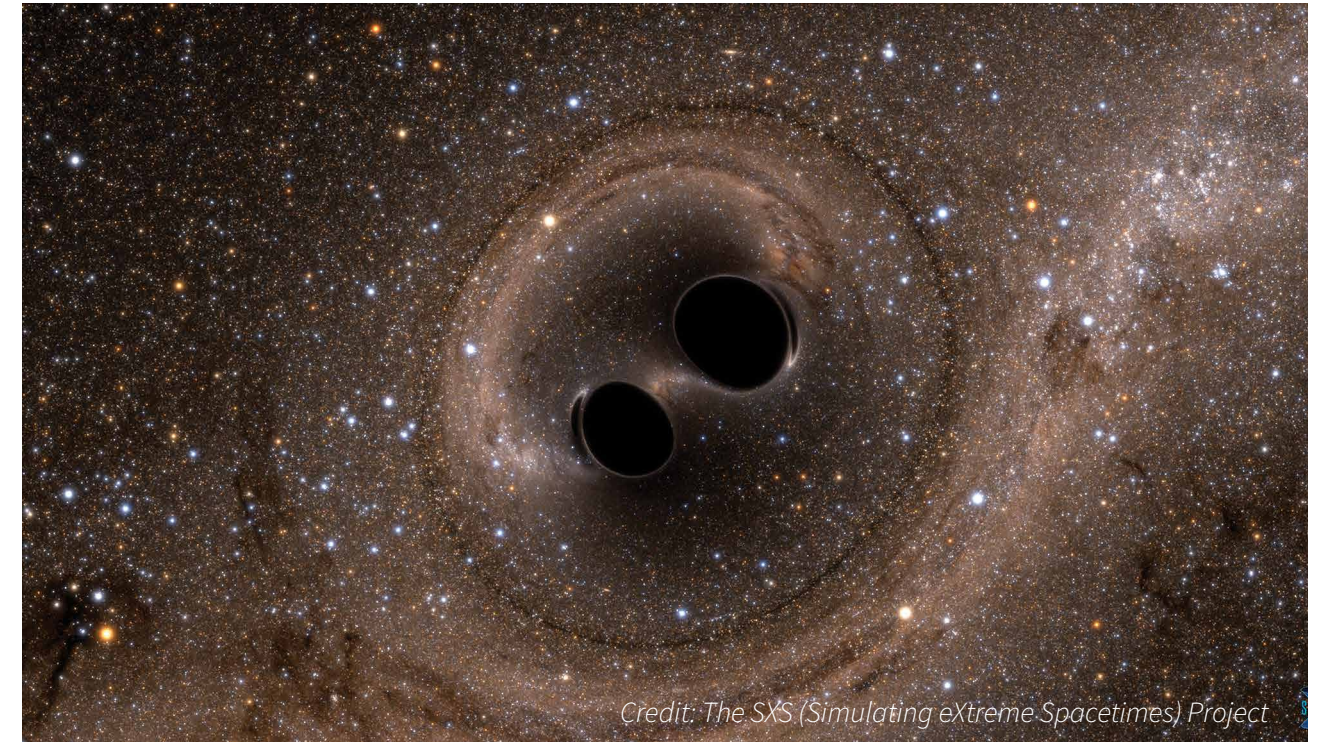


Credit: LIGO lab/C. Gray

On the 14th of September 2015 scientists at the Laser Interferometer Gravitational-Wave Observatory observed ripples in the fabric of spacetime for the very first time. These ripples, known as gravitational waves, arrived at Earth from a cataclysmic event in the distant universe. First predicted by Albert Einstein a century ago, this discovery marks the last remaining test for his theory of general relativity, and opens up a new window into our understanding of the cosmos.

The gravitational waves were picked up by the two LIGO detectors operating in unison – one located in Livingston, Louisiana, and the other in Hanford, Washington. Here, we have had the pleasure of speaking with **Dr Kiwamu Izumi**, a researcher working at the LIGO Hanford site, who tells us all about gravitational waves, the first day they were detected, and what the future holds for this new field of gravitational wave astronomy.

**‘I was not fully convinced whether the detection was real at the beginning. My initial thought was that it could be noise or a synthesised signal injected by someone for testing purposes.’**



Credit: The SXS (Simulating extreme Spacetimes) Project

**Please tell us about the day that gravitational waves were first detected. Did you immediately know that the signal was real?**

The first gravitational wave signal, named GW150914, reached the earth at 2:50 AM on September 14th, pacific time. Several hours later, I woke up and drove in to the Hanford observatory as usual but did not know about the event. Then we had a site-wide meeting at 8:30 to discuss various things. However, interestingly, nobody talked aloud about the event that was detected in the early morning during the meeting although some of us already knew it by the alert and emails. So for me, it was one of those usual days until the mid-morning. After the meeting, one of my bosses came to my office and told my office mate, Sheila Dwyer, and me that there was an interesting candidate event in the early morning so we should immediately check the instrument status at the time of the event. I was not fully convinced whether the detection was real at the beginning. The official observation run had not even started although the instrument had been fully functional with high sensitivity. My initial thought was that it could be noise or a synthesised signal injected by someone for testing purposes. A number of people then spent hours in the afternoon to check the instrument status and confirmed that the instrument had been in a good shape.

**What exactly are gravitational waves, and how they are generated?**

Gravitational waves are ripples of space-time predicted by Albert Einstein a century ago in 1916, as a consequence of his theory of general relativity. In the theory, gravitation is described by the distortion of space-time as opposed to a Newtonian force. General

relativity predicts that gravitational waves can be radiated from a moving mass similarly to electro-magnetic waves radiated by a moving charge. A big difference between gravitational and electro-magnetic radiation is that the amplitude of gravitational waves one can produce in a laboratory is so small that it is extremely impractical to generate and observe them in a laboratory. Instead, to radiate gravitational waves with large amplitude, one essentially needs an astrophysical system with highly accelerated massive objects, for example, the merger of two black holes.

**So how do the LIGO detectors work? And how can they detect space-time deformations that are so infinitesimally tiny?**

LIGO uses a laser interferometer to sense the distortion of space-time. It is essentially the same as the Michelson-Morley experiment in which light is split by a beam splitter and each beam is reflected back by a mirror to the beam splitter, resulting in an interference pattern in the light detected at the other side of the beam splitter. With such a setup, one can measure difference between the distances of two arms caused by the passage of gravitational waves.

A key point is that gravitational waves induce strain in the arm lengths. This means that the longer the arm lengths are the greater the displacement in the arm lengths is. This is the reason why the LIGO interferometer has such a long arm length of 4 km. Ultimately the sensitivity should be limited only by fundamental physics such as quantum and statistical mechanics.





Credit: Caltech/MIT/LIGO Lab

**So far, two gravitational wave signals have been obtained. From analysing the data, how did the staff at LIGO know that black-hole mergers were responsible?**

All that information is encoded in the observed waveforms. A system with two massive astronomical objects orbiting each other generates gravitational waves. The radiated waves exhibit almost a sinusoidal wave form, but with its frequency and amplitude increasing as the two objects keep approaching each other due to the system losing angular momentum and radiating it in the form of gravitational waves.

If one looks at how fast the frequency evolves, the characteristic mass of the system (called chirp mass) can be determined – the more massive the system is the faster the frequency evolves. In the case of the first event, because the chirp mass was estimated to be 30 solar masses corresponding to a total mass of more than 70 solar masses, a system with a pair of neutron stars (each of which is typically 1.4 solar masses) is too light to explain the system. Another possibility, the system with a neutron star and a black hole, would not be able to reach such a small separation of 250 km without contact due to the fact that the size of the black hole would need to be approximately 1000 km in this case. Therefore, the only logical option is a pair of black holes whose masses are approximately 30 solar masses each.

**Can the specific black-hole merger events that caused the signals be directly observed using other means, such as telescopes?**

From astrophysical point of view, the merger of a pair of black holes is thought not to radiate electro-magnetic waves. In fact, a number of telescopes, for variety of wavelengths from radio wave to gamma ray, conducted follow-up observations but none of them found any obvious emission in the region where the gravitational wave sources might be located. Later, Fermi, a gamma ray satellite, reported a potential event coincided with the first event but the validity of this event is still under debate.

**Why do gravitational waves propagate at the speed of light? What is so special about this value, and why can nothing travel faster than it?**

It is hard for me to answer the first part. The best I can say is the following. In space without matter, the general theory of relativity tells us that gravitational waves should propagate at the speed of light.

As for the second half of the question, according to the theory of special relativity, nothing can move faster than the speed of light. This makes the speed of light special. The speed of light can be reached only for those which don't have mass, for example photons or light. If things moved faster than the speed of light it would violate what we call 'causality'.

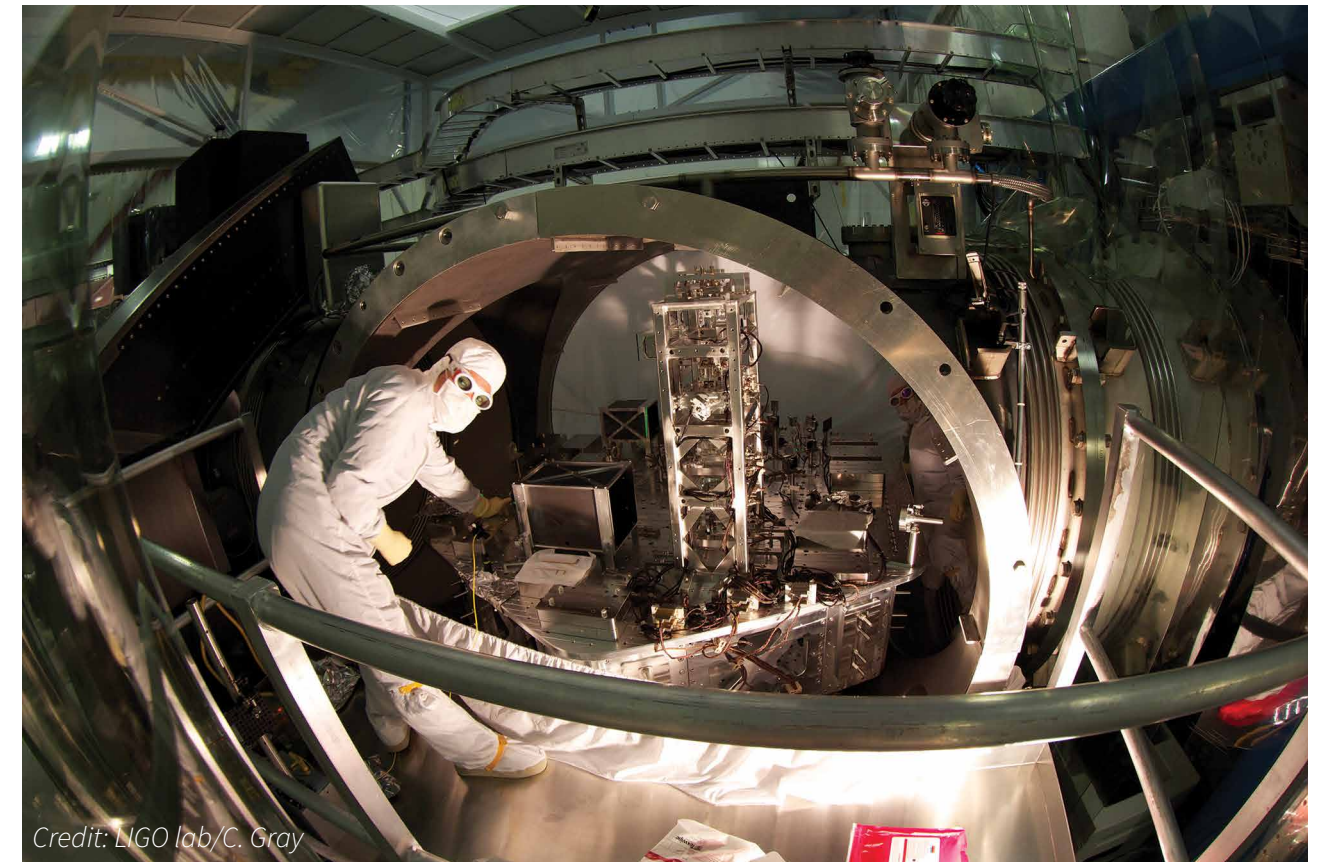
**If gravitational waves can cause matter to squeeze and stretch as they pass through, how come they remain unaffected by matter? Surely this squeezing and stretching will lead to the wave losing energy?**

Technically speaking, yes, gravitational waves lose the energy as they pass through matter. However, the interaction of gravitational waves with matters is extremely small and therefore the energy loss is negligible. This is actually the reason why their direct detection was so difficult. On the other hand, this makes gravitational waves special as an astrophysical observation tool because they are not affected by the matter that the waves encounter during propagation.

**What does the finding mean for physics? Was this the final piece in the puzzle for the theory of general relativity, and is this finding compatible with the theory of quantum mechanics?**

The direct detection of gravitational waves has been thought as the last major remaining test for the theory of general relativity. All other

**‘The direct detection of gravitational waves has been thought as the last major remaining test for the theory of general relativity’**



Credit: LIGO lab/C. Gray

experimental tests for general relativity in the past were for the regime of a weak gravitational field in which other alternative gravitation theories predict little difference. In contrast, the detection of gravitational waves is for the regime of a strong gravitational field for which the use of alternative theories exhibits a noticeable difference. The fact that the detected waveform beautifully matches the prediction from general relativity for the first event indicates that general relativity is correct at the precision level that LIGO has achieved. Looking for small deviations from general relativity in the waveforms will be of great interest in the next decades.

Also, the detection of gravitational waves from merging black holes directly proved the existence of black holes in the universe.

While the existence of gravitational waves itself has little to do with quantum physics, the direct observation of black holes by gravitational waves might serve as unique

proof to study the connection between the gravitational and quantum physics in black holes.

**Now that we know that gravitational waves are a real and measurable phenomenon, what does the future hold for research at LIGO?**

The first thing LIGO will do is bring the sensitivity as close as possible to the design sensitivity. This will increase the detection rate of gravitational wave events and improve the signal-to-noise ratio of individual events. As the sensitivity gradually improves in the coming years, LIGO will conduct two other observation runs. As more events are detected, we will be able to estimate the population of binary black holes more accurately and therefore shed light on the theory of the black hole formation in the universe. In addition, we will be able to detect systems involving neutron stars, for example, binary neutron stars. These

systems will not only give us some insight to the interior structure of neutron stars, but also offer the opportunity for telescopes to look for associated electro-magnetic emissions which should convey a variety of astrophysical information.

Other gravitational wave detectors around the world (such as VIRGO, KAGRA and LIGO-India) will also come online in the coming years. These detectors together with LIGO will form a worldwide detector network to improve the sky localisation ability which is crucial to perform gravitational wave astronomy, a new branch of astronomy that has only just begun.







# DARK IS THE NEW BLACK

Eighty-four percent of the matter in the universe is made of something we cannot see, cannot detect, can only guess at based on the gravitational shadows it leaves in the visible universe. Sounds crazy? Welcome to the world of dark matter, where teams of researchers collaborate to catch this most elusive of prey. Here, we meet the NewDark group, at the Laboratory of Theoretical and High Energy Physics, CNRS, France, whose ambitious quest is to uncover the true nature of this mysterious substance.

We live in a vast and terrifying universe in which our homes and cities are, quite literally, infinitesimal. Our eyes can perceive a tiny fraction of a spectrum which ranges from energetic gamma rays to ground-penetrating sub-radio frequencies; we complain of unseasonably warm and cold temperatures in a climate which makes up a tiny, tiny fraction of a universe ranging from the almost absolute zero of deep space to the million-plus degrees inside stars. And even when we bring out our best equipment, our sensors and telescopes and large hadron colliders, then we still cannot see the majority of the universe. Eighty-four percent of the matter in the universe is hidden, undetectable, seen only by its gravitational interactions with other objects. Known as Dark Matter, this mystery has galvanised the attention of physicists for many, many years.

### Dark what?

You think this sounds interesting, but are unsure as to what, exactly, Dark Matter is? Well, you aren't alone. We asked members

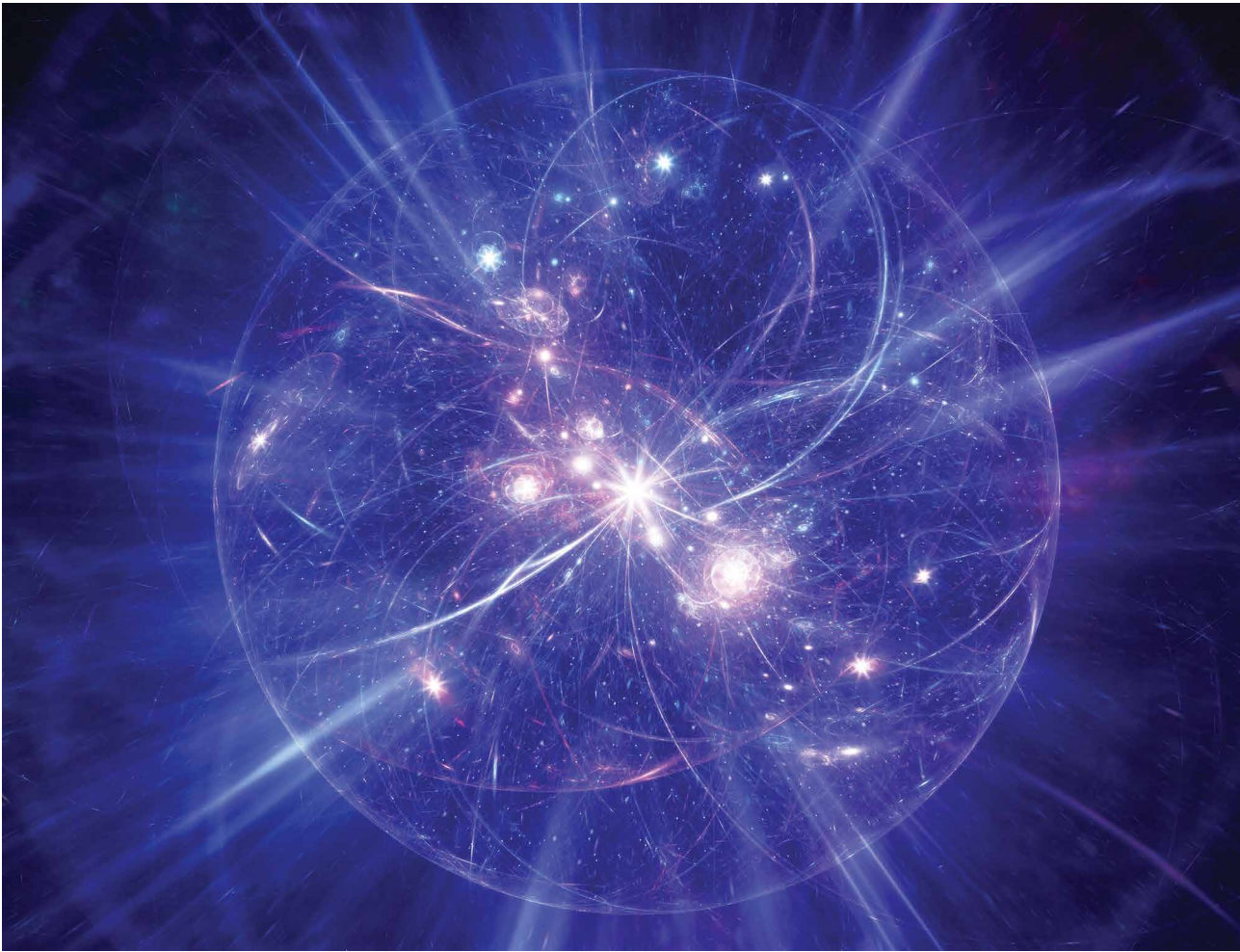
of the NewDark group, at the Laboratory of Theoretical and High Energy Physics in France, who are quite frank about humanity's almost complete lack of knowledge: 'Dark Matter is most probably a particle – as opposed to some sort of fluid – which doesn't interact with light. It is stable, heavy – enough to catalyse galaxy formation – and, last but not least, different in nature from the stuff of everyday matter. A quick check shows that none of the known particles can fit the bill.'

The question then is – how do we know anything about dark matter at all? The first clues popped up in the last century, as astronomers noticed that galaxies were able to spin faster than should be possible – the amount of visible mass simply could not provide enough gravitational force to hold everything together. More detailed experiments followed, studying stellar movement and gravitational lensing, in which gravity bends the light of stars behind the object to act as a colossal magnifying

glass. The gravitational effects of dark matter can be seen far from the visible stars and gases we are used to; indeed, dark matter forms a vast network throughout space which we can detect from measurements of the cosmic microwave background radiation. Dark matter filaments (if we can use a word so dainty for macrostructures larger than our galaxy) and their crossings seem to have acted as gravitational cores to seed the very starting locations of galaxies themselves.

So we know that it is out there, we can see the shadows which dark matter casts in our safely visible space-time pond. However, as the NewDark team know too well, the very same features that make Dark Matter cosmologically important also make it extremely difficult to detect. Many attempts have been made to spot the offshoots of dark matter – highly sensitive detectors buried deep in the earth watch for the slightest spark of interacting particles, satellites in high orbits watch for the curious fluxes of subatomic particles associated with dark matter annihilations or decay, vast

**'The ultimate goal, as a part of the larger global effort with a long timescale, is the identification of the nature of Dark Matter'**



accelerators smash particles together at very respectable percentages of the speed of light. All glimpse at the hidden features of dark matter, none have provided definitive proof.

### A matter of shadows and gravity

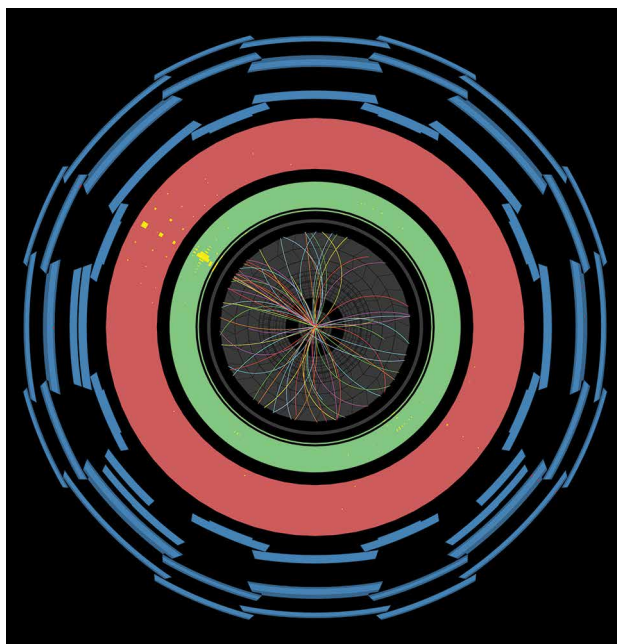
All our experiments have shown pieces of the many-faceted puzzle that is dark matter. Clever interpretation of the available evidence has led to the deductions that dark matter must be a particle (as the density of dark matter decreases as the universe expands), stable enough to last for billions of years without decaying, heavy enough to cluster gravitationally, yet weak enough to barely interact with itself or the other 'standard' particles in the universe. A complex set of properties, which cannot be fulfilled by the particles we know of at the moment.

So what could dark matter be? Physicists have thought up many ideas for dark matter particles over the years. Many of these particles fall under the broader category of 'WIMPs' (Weakly Interacting Massive Particles). WIMPs are so named for their meagre interactions with the 'standard' atoms and subatomic particles we are used to, passing in their millions through each of us with barely a twitch of interaction. Theoretical extensions to the standard model of particle physics independently predicted a WIMP particle whose properties matched calculations derived from dark matter studies, a fact which managed to excite a number of physicists – at least until results from the Large Hadron Collider seemed to put the idea under tension. This was unfortunate both for the resulting disappointment and for the fact that one of the many hypothesised candidates was the

*higgsino* – the super-symmetric partner of the Higgs boson. The Higgs boson is often hyperbolically referred to as the 'god particle' in the media, which would theoretically make the *higgsino* somewhat satanic – perfect for those aspiring sci-fi/horror writers out there.

Luckily dark matter research is in no way short of excellent names. Directly contrasting the WIMPs is the MACHO hypothesis (for massive astrophysical compact halo object). In this hypothesis, dark matter is actually just standard matter in forms which we can't see (brown dwarves, neutron stars, special black holes and the like), although recent work has indicated that there cannot be enough of these objects to make up all the missing matter. What else? We could try looking for axions, slow moving hypothetical particles with a low mass (in contrast to the 'massive' WIMPs), which could theoretically





ATLAS Experiment © 2016 CERN

**Dark matter could be made of particles 1000 times heavier than the proton or as light as a millionth of an electronvolt, or of sterile neutrinos, cousins of the known neutrinos but insensitive to ordinary forces. Or even more exotic possibilities.**

be detected as they transmutate into photons. Or we could embrace the Kaluza-Klein theories, which bring us the concept of a 4th spatial dimension (in addition to length, width and height), which is curled up at sub-atomic scales in a way which we could never detect, save for the particles left behind as they decay and the gravitational interactions they leave behind themselves.

The problem, however, is that none of these particles have actually been detected. Even multinational collaborations consisting of billions in funding such as the Large Hadron Collider have so far failed to detect signs of these hypothesised pieces of exotic matter. In the words of Dr Marco Cirelli, the head of the NewDark group, this lack of success has allowed 'theorists' minds to start wandering in less explored territories. Dark matter could be made of particles as light as a millionth of an electronvolt, or of sterile neutrinos, cousins of the known neutrinos but insensitive to ordinary forces. Or even more exotic possibilities.'

#### Even more exotic?

More exotic than the reflections of tightly wound-up higher dimensions? Welcome to the world of theoretical physics and dark matter! The NewDark project was set up in an effort to make some sense of this field, by bringing together expertise from the multiple overlapping fields involved in dark matter research. The project covers

theory, the particular physical signals detected from particle colliders, direct and indirect methods, and astrophysical observations. The hope is that by blending the information found in multiple different fields, with their associated strengths and weaknesses, a clearer picture of the mysterious particle will be formed.

The Laboratory of Theoretical and High Energy Physics, where the NewDark group is based, is a joint research venture between the French national research body known as the Centre National de la Recherche Scientifique (CNRS) and the renowned University Pierre et Marie Curie, one of the largest scientific complexes in France. Its overarching goal is to conduct research into quantum field theory, a method of modelling all subatomic particles (from electrons to quarks) as excitations of an underlying 'quantum field.' These approaches allow us to explain the strange manner in which subatomic particles are able to act as both a discrete particle and a continuous wave *at the same time*.

#### Energetic researchers in a field of data

The aims of the NewDark project fit perfectly into this overarching goal, with their scientific interests and varied backgrounds allowing them to effectively study the many facets of dark matter research. The NewDark group is highly international, in keeping with physics research itself, having members from Italy, France, England, Greece, Switzerland and Germany. Born in Italy but based in Paris for over 10 years, Dr Cirelli has a long history of high-energy physics behind him, a history which has slowly led him over the last few years into the complex world of dark matter. He is supported by several postdoctoral researchers, including Dr Mathieu Boudaud (who is examining cosmic rays for signs of dark matter interactions); Dr Bradley Kavanagh (who follows results from dark matter signal detectors buried deep under the ground); Dr Kallia Petraki (who studies the microscopic nature of dark matter interactions); and Dr Filippo Sala (who covers both theoretical and experimental aspects of particle physics). Previous members of the team include Dr Marco Taoso and Dr Gaëlle Giesen, both experts in the field of dark matter research. The group also assists in the training of up-and-coming scientists, with PhD student Marta Perego rounding out the group – her focus lying in the field of particle colliders and associated dark matter production.

This sounds like a lot of different methods for one group, but Dr Cirelli is convinced that it is the only practical approach. 'A multi-faceted attack at the problem is necessary,' he muses, 'because the tools at our disposal are powerful but only partial. For instance, the LHC is a machine which, in its first years, will not easily provide precision measurements; the astroparticle experiments, by nature, are plagued by astrophysical backgrounds. Only via cross-correlating partial information from different fields will progress be made.' Their hope is that these multiple avenues of research will lead to better results than specialising in one field alone.

'The ultimate goal,' comments Dr Cirelli, 'as a part of the larger global effort with a long timescale, is the identification of the nature of the Dark Matter.' No minor goal this one, but given the quality of the team on the case, one with potential.

# Meet the researchers



## Marco Cirelli

Dr Marco Cirelli obtained his PhD from Scuola Normale Superiore in Pisa and went on to perform postdoctoral research at Yale University, CEA Saclay and CERN. Since 2015 he has held a position at the Laboratory of Theoretical and High Energy Physics at Sorbonne University in Paris. In 2011 he was assigned a Starting Grant from the European Research Council for the NewDark project: 'New Directions in Dark Matter Phenomenology at the TeV Scale', and in 2014 he was awarded the Prix Thibault from the Academy of Arts and Letters in Lyon. In the last few years his research revolves around solving the Dark Matter problem. He enjoys the challenge and the excitement of working at the intersection among particle physics, astrophysics and cosmology, juggling between the infinitely small and the extremely large frontiers of our current fundamental understanding of the Universe.



## Mathieu Boudaud

Dr Mathieu Boudaud received his PhD in 2016 from University of Savoie in France. During the fall of 2016, he moved to the Laboratory of Theoretical and High Energy Physics in Paris where he currently works as a postdoctoral researcher. Dr Boudaud's research focuses on the propagation of charged cosmic rays in the Galaxy and on the indirect detection of astrophysical dark matter from antiparticle cosmic rays.



## Bradley Kavanagh

Dr Bradley Kavanagh obtained his PhD in particle astrophysics from the University of Nottingham in 2014, and soon after moved to Paris to join the NewDark group as a postdoc. Dr Kavanagh's current focus is in studying possible dark matter signals in dedicated underground detectors. He aims to understand what information about the particle physics and astrophysics of dark matter could be extracted from a future signal. A key aspect of this lies in studying how different models of dark matter might be distinguished from one another. Bradley is also interested in looking for new 'Smoking Gun' signatures of dark matter, aiming to open up new directions in the hunt for these ever-present but ever-elusive particles.



## Kallia Petraki

Dr Kallia Petraki received her PhD from the University of California Los Angeles in 2009, where she investigated possible connections between dark matter and neutrinos. She continued working on dark matter as a postdoc, at the University of Melbourne and at the National Institute for Subatomic Physics in the Netherlands. She is currently a researcher at the Laboratory of Theoretical and High Energy Physics in Paris. In her work, she aims to understand the microscopic nature of dark matter, and in particular how the fundamental properties of dark matter may reveal themselves in the way our universe is structured.



## Filippo Sala

Dr Filippo Sala obtained his PhD in physics at Scuola Normale Superiore, Pisa, and has been a postdoctoral researcher in the NewDark group, since December 2013. Before joining the group, he was also a long term visitor at CERN and the Lawrence Berkeley Laboratory. He is curious about how Nature works at a fundamental level, at the smallest and largest scales of length and time. These domains still hide the answers to some fascinating mysteries, like what constitutes dark matter. Dr Sala's research activity explores theoretical ideas that address these issues, for example by studying how they can be best probed using current and future collider experiments, as well as using telescopes. His approach is characterised by the cross-fertilisation of a broad set of neighbouring domains, in both particle and astroparticle physics.



## Marta Perego

Marta Perego is currently a PhD student in particle physics at CEA Saclay. She is part of the ATLAS collaboration of the Large Hadron Collider at CERN, where her main interest is searching for dark matter production at colliders. She works both with the ATLAS group and with NewDark on collider dark matter searches. She performs data analysis to look for dark matter produced in vector boson fusion processes in the high energy proton collisions collected by the ATLAS detector in 2015 and 2016.



## Marco Taoso

Dr Marco Taoso is an Italian physicist working on dark matter, astrophysics and particle physics. After his PhD, obtained from University of Padova in Italy and in co-tutorship with Université Paris Diderot, he has held several post-doctoral appointments. In 2013, he moved to Paris, to join the NewDark group. As well as helping him to find new ideas and interesting directions for his scientific career, his post with the NewDark group was also a lot of fun for many reasons: friendly collaborators, a chance to live in Paris, delicious macarons and chocolate tastings to brighten up workshops and scientific meetings, as well as travelling to wonderful and exotic places to attend conferences! Now, Marco Taoso is working at the Instituto de Fisica Teorica in Madrid, Spain.



## Gaëlle Giesen

Dr Gaëlle Giesen graduated from École polytechnique fédérale de Lausanne with a Master's degree in Physics, and soon after joined the NewDark group in 2012. Here, she completed a thesis on indirect detection of Dark Matter using charged cosmic rays and received her PhD from the University Paris-Sud in 2015. Also passionate about space technologies, she is now working at the launchers directorate at the Centre National d'Etudes Spatiales (CNES). Photo credit: Gislain Mariette





# INVESTIGATING PLASMA STORMS AND SUBSTORMS IN OUR NEAR-EARTH BACKYARD

Space physicist **Dr Tony Lui** has spent four decades increasing our understanding of the mechanisms behind the magnetic disturbances and interactions of space plasma surrounding our home planet.

## Plasma, a ubiquitous state of matter

In the calm and comfort of a classroom, we have all learned about atoms and molecules. We think of these tiny constituents of all matter as having a compact nucleus with a peaceful cloud of orbiting electrons. The positive charge of the nucleus and the negative charge of the electrons sum to zero and life is neutral and good. But what if those atoms and molecules exist in regions of space where temperatures are, well, *astronomically* high – hot enough to strip these electrons from their nucleus? Then we do not have the tame, neutral atoms and molecules we are familiar with on Earth – we have plasma.

Plasma – the so-called fourth state of matter, is a gas so hot that its atoms are split into a cloud of ions and electrons that move and flow independent of each other. Astronomically speaking, plasma is everywhere. Our sun and the stars are made of plasma, for example. The solar wind and the tails of comets contain plasma. Even in our everyday experience here on Earth, plasma can be found in such places as fluorescent and neon lights, lightning flashes,

candle flames and the cores of nuclear reactors. Overall, roughly 99.9% of the matter in the Universe exists as plasma, rather than the solid, liquid or gas we know here on Earth. And because the particles that make up plasma are electrically charged, they can conduct electricity and are influenced by magnetic fields. This makes for fascinating electromagnetic interactions, some of which Dr Lui has studied for most of his professional life.

## The Earth's Magnetosphere – a plasma playground

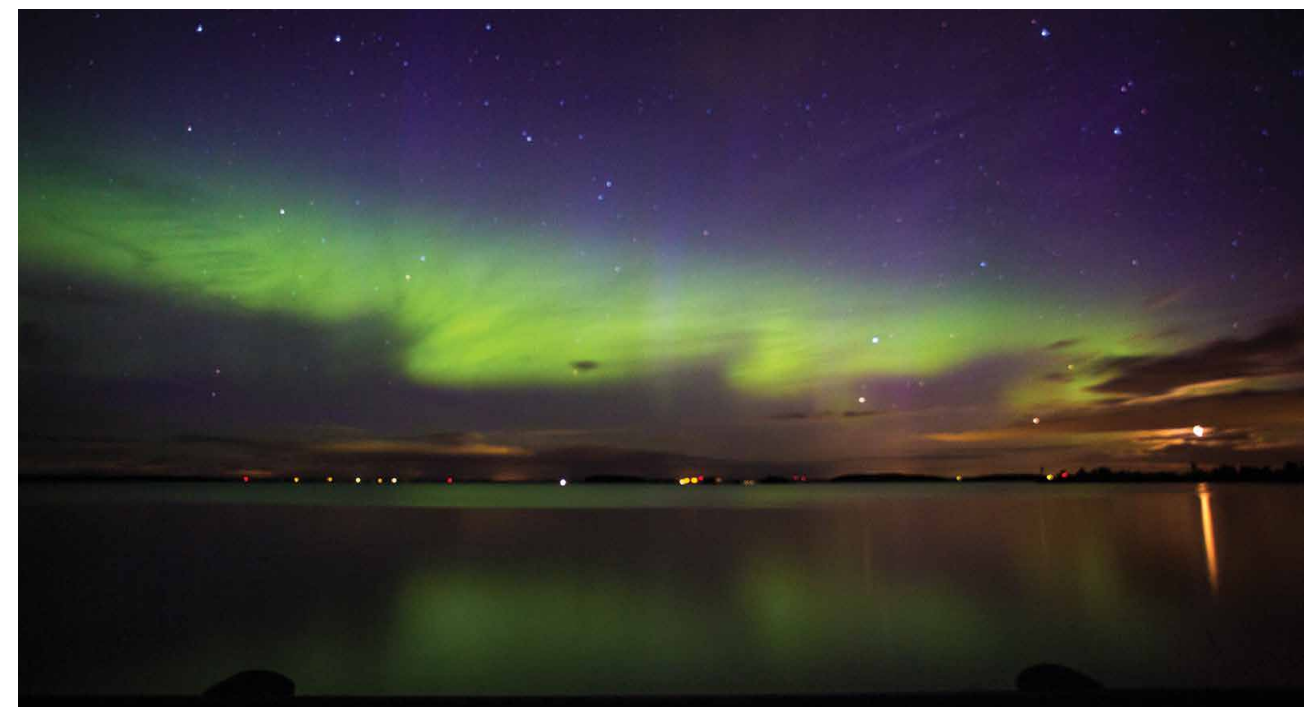
The Earth, because of its liquid outer core of molten iron, is surrounded by an intrinsic magnetic field. Thus, we describe the Earth as having a north 'pole' and south 'pole', just as we would a magnet. The space surrounding the Earth where its magnetic field significantly influences the plasma contained there is called the *magnetosphere*. Most of this plasma comes from the Sun in the form of the solar wind.

The Sun, or any star for that matter, gives off massive amounts of plasma in what is called a stellar wind. For our Sun, it's termed the

*solar wind*. This hot, ionised gas travels out from the star and interacts with any objects it encounters, particularly those with magnetic fields. Thus, the Earth's magnetosphere interacts with the solar wind as does the magnetosphere of Jupiter or Mercury or any other planetary body with a magnetic field. On the impact side of the planet – the planet's 'day' side – there is a significant compression of the planet's magnetic field by the solar or stellar wind, especially at high altitudes. On the 'night' side of the planet – with the solar wind blowing on past – the plasma flow actually causes the magnetosphere to stretch out into a 'tail', conceptually similar to the tail of a comet.

Here on Earth we see some of the interaction between the solar wind and the Earth's magnetosphere, especially in the northern and southern latitudes. Most commonly, the interactions result in spectacular light shows called *aurorae*. In the northern hemisphere, this is called the *Aurora Borealis* – the Northern Lights, while in the south the same phenomenon is known as *Aurora Australis*. But all consideration of visual spectacle aside, the fact that high energy ionic particles are dancing around our near-Earth space

**'This research has potential applications in understanding the physical processes responsible for many explosive phenomena in the universe at large'**



interacting with the Earth's magnetosphere leads to a very practical question: what effect does all this have on our space objects, such as telecommunication satellites or the International Space Station? We know that solar flares, or sun spots, dramatically increase the solar wind at intervals, and may at times disrupt our communications. How can we predict that or prevent it? What about the effects on our orbiting electronics and people? We need to know how all this works so we can find answers to these important questions. And that is what Dr Lui has worked on for years, investigating the physical processes of near-Earth plasma disturbances to hopefully allow forecasting of space disturbances as we achieve a better understanding of their underlying causes.

## Let's start by watching the light show!

Dr Lui tells Scientia about what inspired him to dedicate his career to the study of space phenomena. 'The year I graduated with my physics degree from the University of Hong Kong was the year that the U.S. had the

Apollo 11 lunar landing with Neil Armstrong as the first human stepping on the surface of the moon,' he explains. 'He left "the next giant leap imprint". I was attracted to space research by this exciting adventure.'

Soon after this, some of his early research which was published in 1973 in *Planetary Space Science* interpreted data from the Canadian satellite ISIS-2, one of a series of Canadian satellites launched to study the Earth's ionosphere. ISIS-2 was able to take images of the entire Aurora Borealis from above. Dr Lui analysed the resulting images and discovered that, unlike images taken from the ground, the big picture from above indicated that the aurora was not individual auroral arcs as depicted in popular photographs. There was actually a diffuse and uniform belt of aurora that contained some discrete arcs and bands encircling the poles. This was a significant leap in our understanding of auroras and considered a major achievement in our understanding of the solar-terrestrial relationship.

A few years later, while studying auroral images from Defence Meteorological Satellite Program satellites, Dr Lui described large-scale, saw-toothed undulations on the equatorward edge of the visible diffuse aurora during magnetic storms. He found that the amplitude of these waveforms was large, from about 40 to 400 km, which would explain why it had not been observed from the ground. This phenomenon could last for about 0.5 to 3.5 hours. In each of the four cases he observed, the undulation occurred during a geomagnetic storm interval near the peak development of the storm time ring current. This finding was exciting enough to be reported in the local newspaper and it was later published in the *Journal of Geophysical Research*.

As technology progressed, Dr Lui kept progressing as well, particularly since satellite auroral imagers were continuously being improved with each new satellite mission and global views of auroras became more abundantly available for investigation. This allowed researchers like Dr Lui to conduct



large analyses on auroral images to examine the dynamic behaviour of auroral activities – hence also magnetospheric activities – in an unprecedented way.

Getting a bird’s-eye view of solar storms

Early on, it was known that the worldwide depression of the geomagnetic field during magnetic storms was caused by the development of a ring current encircling the Earth. However, because of the lack of measurements covering the energies for the ring current, determination on the evolution of the ring current during a magnetic storm was lacking until the Active Magnetospheric Particle Tracer Explorers (AMPTE) mission was launched – three spacecraft designed to study the sources, transport, and acceleration of energetic magnetospheric ions. This was the first time that the evolution of the ring current during two magnetic storms was quantitatively investigated. Dr Lui calculated from the data that the ring current intensified initially at its outer edge and later evolved to cover over the entire radial range of the ring current. This was published in 1987 in the *Journal of Geophysical Research*.

Perhaps even more exciting, a new diagnostic tool for magnetospheric investigation was developed for looking at energetic neutral atom (ENA) emissions, produced by charge-exchange collisions between energetic magnetospheric ions and the cold neutral hydrogen atoms. ENA measurements were taken with the Geotail satellite, a joint Japan-USA endeavour to study the structure and dynamics of the tail region of the magnetosphere with a comprehensive set of scientific instruments. Prior to the Geotail mission, no composition information for ENA emissions was available. The first composition information of ENA was reported with measurements from the Energetic Particles and Ion Composition (EPIC) instrument on Geotail. Dr Lui published some of the first composite measurements of ENA in *Geophysical Review Letters*. Further, he combined ENA data of the global magnetospheric population with ground-based observations by the networks of radar to solve an ongoing controversy about the cause of storms – were they caused by smaller, substorms building to a crescendo and resulting in a full-sized storm, or was it an increase in the convection of plasma from the tail enhancing the current ringing the Earth that ultimately led to storm development? He found that solar storms basically had both causes, one from enhanced convection without smaller substorm activity and the other from substorm activity with even a decrease in convection. Therefore, Dr Lui resolved the controversy by showing that both causes can lead to storm development independently. This novel insight was published in *Geophysical Research Letters*. ‘The research undertaken has implications on space weather forecasting that can affect space assets as well as societal functions such as high-altitude flights and power blackouts due to space disturbances,’ Dr Lui tells Scientia. ‘It also has potential applications in understanding the physical processes responsible for many explosive phenomena in the universe at large.’

Where is Dr Lui looking now? To the sky, of course

It helps to be connected, and Dr Tony Lui is that. As a recognised expert in near-Earth space physics, he has his fingers in many projects. Right now he has a number of irons in the fire to continue his search for the whys and wherefores of our magnetosphere.

He is still a principal investigator in the monitoring of the Energetic Particles and Ion Composition (EPIC) instrument on the Japanese-



NASA satellite Geotail in the International Solar-Terrestrial Physics Science Initiative (ISTP) program. It was launched in 1992 and the data is still flowing in. Dr Lui is also a co-investigator of the Research with Adaptive Particle Imaging Detectors (RAPID) instruments on European Space Agency’s Cluster satellite mission, launched in 2000. The aim of that instrument was to record data to generate 3-dimensional distributions of high-energy electrons and ions in plasma.

**‘The evolution of electrical currents in space plasma can be revealed in future suitable satellite constellation, which will yield very illuminating clues on the nature of physical processes that produce explosive energetic phenomena and turbulence in space plasmas.’**

In 2007, NASA launched a set of five satellites named THEMIS – Time History of Events and Macroscale Interactions during Substorms. The purpose of those satellites was to study substorms in the Earth’s magnetosphere, especially where they intensify auroras near the poles. Three of the satellites remain in the magnetosphere, while two have been restationed near the Moon. Importantly, the satellites record their data from space and correlate it with ground-based imagers and magnetometers. Dr Lui is a co-investigator on the THEMIS project, as well as on the NASA Van Allen Probes, two satellites taking measurements of goings-on in the Van Allen radiation belt. Understanding activities of plasma in the Van Allen belt is important in understanding the behaviour of plasma elsewhere in the Universe.

Another ongoing satellite project Dr Lui with which is associated is the Fast Auroral Imager on the Canadian Space Agency’s CASSIOPE satellite. The satellite carries a scientific experiment package called e-POP (enhanced Polar Outflow Probe) that gathers data on solar storm effects in the upper atmosphere that can interfere with GPS and other communications. This is precisely what Dr Lui’s research is aimed at – understanding solar weather and eventually devising measures to mitigate its deleterious effects.

What’s next for Dr Lui?

Dr Lui hopes that the next steps for his research would lead to additional understanding of the phenomena of magnetic storms and substorms and finding evidence for the underlying physical processes for these phenomena. In particular, explanation of the evolution of electrical currents in space plasma can be sought in future suitable satellite observations. ‘This will yield very illuminating clues on the nature of physical processes that produce explosive energetic phenomena and turbulence in space plasmas’ Dr Lui explains. In all, Dr Lui has enough work to keep him busy for a further four decades!



Meet the researcher

**Dr Tony Tat Yin Lui**  
Senior Professional Staff Scientist  
The Johns Hopkins University Applied Physics Laboratory  
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Dr Tony Tat Yin Lui received his PhD in Space Physics in 1974 from the University of Calgary. In 1979, after undertaking postdoctoral fellowships in Physics at the University of Calgary and Geophysics at the University of Alaska, as well as a Research Associate stint at the Herzberg Institute of Astrophysics, NRC of Canada, Dr Lui joined the Space Department at The Johns Hopkins University Applied Physics Laboratory.

Currently Dr Lui is Principal Investigator or Co-Investigator of several on-going space missions, including the Japanese satellite GEOTAIL in the ISTP program, the European Cluster satellite mission, the NASA Explorer mission THEMIS (Time History of Events and Macroscale Interactions during Substorms), the NASA satellite mission Van Allen Probes, and the Canadian satellite e-POP. He has edited seven scientific books and published more than 360 scientific papers in refereed journals. One early publication was cited by the American Society of Physics as one of the twelve outstanding pieces of research in Physics for 1973. Another publication in the same year was highlighted in Moldwin’s Timeline of Solar-Terrestrial Physics. Dr Lui has received several NASA and ESA Awards and was elected to be a Fellow of the American Geophysical Union in 2008.

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FUNDING

NATO  
National Science Foundation  
NASA

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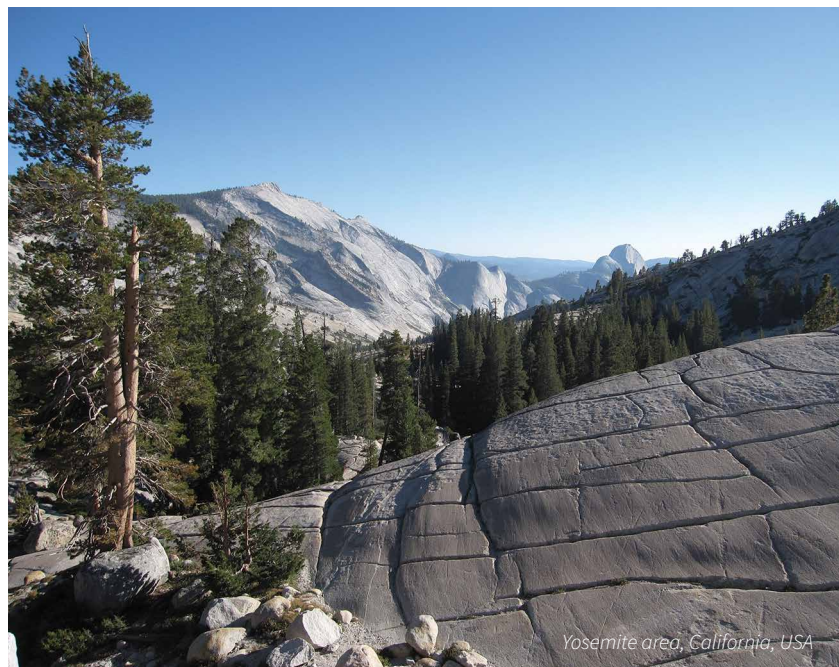


# USING RADIOISOTOPES IN VOLCANIC CRYSTALS TO MEASURE THE AGE OF THE EARTH

**Professor Urs Schaltegger**, with his colleagues in the Isotope Geochemistry Group at the University of Geneva in Switzerland, analyse isotopes of Uranium and Lead found in crystals of the mineral zircon to determine the age of geologic deposits. They use this data to determine the time frame of geologic catastrophes such as mass extinctions.

Every child has asked a grandparent: ‘How old are you?’ Often the answer laughingly is: ‘I’m as old as the hills!’ Of course, this is a loose reference to a verse in the Bible in the book of Job: ‘Art thou the first man that was born? Or wast thou made *before the hills?*’ It follows, naturally, that the next question the child might ask is: ‘Well, then, how old are the hills?’ To answer that requires more than the Bible – we need science. A good place to turn to for that answer is Professor Urs Schaltegger and his colleagues in the University of Geneva’s Department of Earth Sciences. They utilise the modern science of geochronology to pinpoint the exact age of ‘the hills’.

Geochronology is the branch of science dealing with the age of rocks, sediments and fossils by studying chemical or physical properties of the materials themselves, rather than characteristics of the surrounding area. We commonly think of determining the age of a dinosaur bone or bones of an extinct hominid by looking at the layers of sediment in which we find it – the deeper the layer, the older the bones. But geochronologists want to analyse the material itself to determine the age. Analogous to archaeologists’ radiocarbon dating of prehistoric plant and animal material by determining their level of carbon-14, Professor Schaltegger and his group dates rocks and geologic materials by measuring the decay of radioactive uranium into lead.



Yosemite area, California, USA

## Estimating the Age of the Earth from Radioactive Decay

For over three centuries, scientists have attempted to determine the age of the Earth. Initially, they studied proxies of the Earth’s age, such as the salinity of the seas. As time passes, more material – including salt – washes into the oceans, making them saltier. If we back calculate from the rate of increase in sea salt, we can estimate how

long this has been happening. Clearly this is a bit dicey. When did the first ocean form, anyway? How salty was it to begin with? Was the rate of erosion always the same? Certainly we would like something more accurate. But after the French scientist Henri Becquerel discovered radioactivity in 1896, whole vistas of science opened up. And in 1911 a pioneer in geochronology, British geologist Arthur Holmes, conceived the idea of measuring the decay products of uranium in geologic material to determine the age of

‘Knowing the time allows us to understand geological processes. With our understanding we are able to link processes in the deep earth to what’s going on at the surface.’



Adamello, northern Italy

the material. Specifically, Holmes looked at the radioactive decay of isotopes of uranium into lead isotopes in geologic specimens and used the known half-life of the different uranium isotopes to calculate the age of the geologic specimen. This half-life countdown of uranium actually includes two nuclear clocks:

Uranium is found in nature in two different isotopic forms, uranium-238 and uranium-235. Uranium-238 naturally decays into lead-206. This decay has a half-life of 4.468 billion years, or, as the scientists say, 4.468 gigaannus (Ga). Uranium-235 decays into lead-207, with a half-life of 704 million years, or 704 megaannus (Ma). Using both of these countdown clocks, scientists like Professor Schaltegger can measure the age of geologic material with very good

precision and accuracy, depending upon the concentrations and purity of uranium and lead in the material. Professor Schaltegger and his group at the University of Geneva are learning to read these countdown clocks to more accurately reconstruct the timing of major processes in the geologic history of the Earth.

## Where in the World is the Uranium?

To be able to accurately use uranium-lead (U-Pb) dating, you first have to have geologic material with appropriate amounts of uranium. Scientists have found that the favourite material is the mineral zircon, a zirconium silicate, or  $ZrSiO_4$ . This mineral crystallises over time in cooling magma, deep below the Earth’s surface. During these processes, zircon not only

incorporates atoms of zirconium and silica, but also impurities – among them uranium. Looking into this at the nanometre scale (a nanometre is a billionth of a meter –  $10^{-9}$  m) we can see that molecules within the zircon crystal lattice can be replaced by other molecules, thereby including traces of other compounds in the lattice. One of those impurities that perfectly fits into zircon is *coffinite*, the uranium silicate  $U_2SiO_5$ . Uranium is therefore found as an impurity, or a so-called ‘trace element’, in our zircon crystal. Importantly, what is *not* easily included as an impurity in the zircon crystal is lead – it just hasn’t the right size to fit in the zircon lattice. So zircon is an ideal material that forms in the Earth with a little uranium, but almost no lead. This means any lead found in zircon millions of years after its formation wasn’t there to begin with. It must be a daughter



product of the radioactive decay of uranium. Scientists like Professor Schaltegger can then use advanced modern technologies of isotope separation, using mass spectrometers, to measure the ratios of uranium isotopes and lead isotopes. Since we know the half-lives the isotopes, we can calculate how long ago this specimen of zircon crystallised. This is the basis for U-Pb dating.

Going back to our sea salt analogy, the erosion rate by which salt is washed into the sea can be thought of as our radioactive decay constant, while the initial salt concentration in seawater is equivalent to the initial lead concentration at the moment of zircon crystallisation.

U-Pb dating techniques can be applied to a large variety of geologic problems where the durations and rates of processes need to be precisely and accurately known. ‘By knowing the process rates, we can conclude on the nature of processes and judge whether models are realistic or not,’ explains Professor Schaltegger.

For example, if you obtain and date samples of zircon from different rocks of an area with magmatism and volcanism, you may be able to reconstruct the fluxes of liquid magma from the Earth’s interior to the surface and this would give us a picture of the dynamics of the magma flow even though it happened millions of years ago. This information could be crucial to estimate the risk of volcanic eruptions in a certain area, or how much a magma would have been capable of forming an ore deposit, for instance one of gold or copper. If we know how old the zircon is in one area – basically, when it initially crystalised from the liquid magma – we can contrast that with the age of zircon at another site. Eventually, we might be able to draw a picture of how magmatism, volcanism, rock uplift, erosion, and even topography, evolved in response to plate tectonics.

On a planetary scale, being able to date the oldest zircon crystals of our planet allows us to measure when the earliest materials condensed when the Earth started to cool.

The long half-life of uranium-238 particularly allows us to look back literally hundreds of millions to billions of years. For Professor Schaltegger, some of the interesting aspects of our Earth’s history are periods of profound disturbance of life, climate and environment during the history of the Earth, popularly termed *mass extinctions*. ‘The geological record often does not allow us to prove the causality between two processes directly, a prime example being mass extinctions and volcanism,’ he tells us, ‘but we need to make the detour via age determination. Since geology is a “historical” science that tells the story of the earth, time is essential.’

**Mass Extinctions are Unusually Hot Topics!**

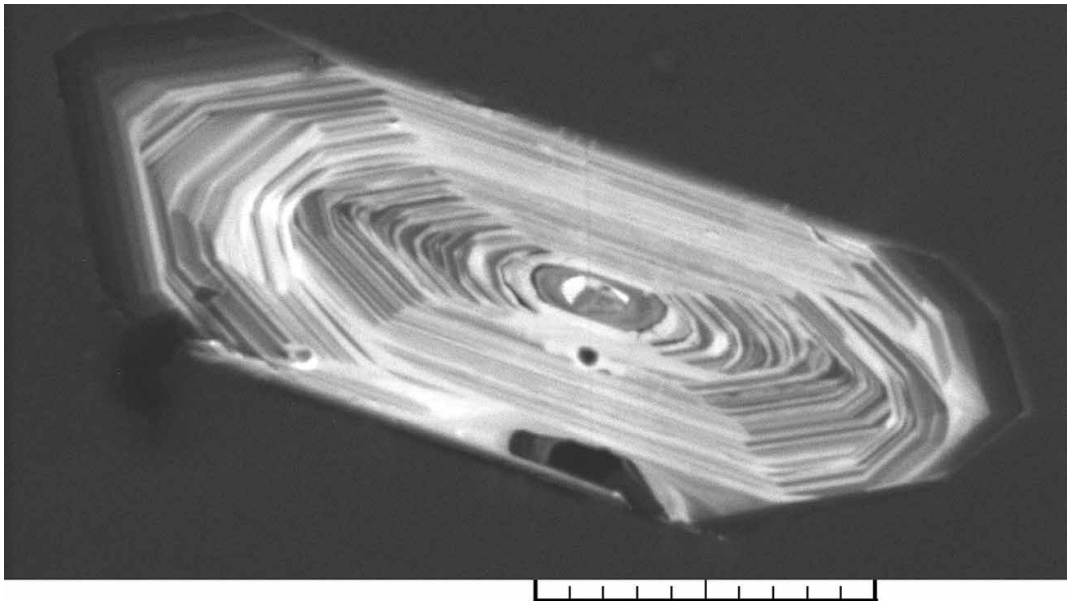
Mass extinctions are intervals of widespread and rapid decrease in life forms on Earth. Since microscopic life is not easy to see in fossils and other geologic material, we usually talk about a decrease in multicellular life, namely animals and vegetation. Mass extinctions in Earth’s history were associated with massive volcanic activity. Most people are familiar with the Cretaceous-Paleogene extinction of the dinosaurs 66 Ma ago, presumably due to the onset of a several hundred thousand year-long period of intense volcanism in the present-day area of the Deccan traps in India. This is the event that is thought to have killed off roughly three-quarters of animal and plant life on Earth. It seems that the environmentally rough conditions additionally



**The important conclusion from the team’s analysis is that it was volcanic activity that caused massive shifts in life on Earth.**

degraded with the impact of the 10 km diameter asteroid or comet that created the Chicxulub crater in the Yucatan peninsula in Mexico.

The temporal coincidence between mass extinctions and periods of massive volcanic eruptions serves as an argument for linking global climatic and environmental catastrophe with the sudden injection of large volumes of gases – such as carbon dioxide, methane or sulphur dioxide – into the stratosphere. These gases are either directly released from decompressing magma when it flows into the upper crust and flows onto the terrestrial surface, or they may be derived from extreme heat – sometimes over 1000°C – acting upon organic-rich sedimentary rocks closer to the surface. As such, these events resulting from volcanism are ideal targets for Professor Schaltegger’s U-Pb dating strategy. Volcanoes means magma and lava, containing tiny crystals of zircon. Zircon means we have a countdown clock for dating purposes. In fact, Professor Schaltegger and his team, along with collaborators in Europe and overseas, are studying important mass extinction intervals in the history of the Earth, such as the boundary between



*Cathodoluminescence images of a zircon crystal that shows how this mineral is growing in a magmatic liquid by adding layers of crystallised material. The difference in greyscale is indicating different chemical composition (black = much uranium; light = much Yttrium)*

the Permian and Triassic 252 Ma ago, the Triassic–Jurassic extinction event, marking the boundary between the Triassic and Jurassic periods 201.3 Ma ago, and the Pliensbachian-Toarcian event – a global crisis reflected by oxygen depletion in global ocean water – marking the end of the Pliensbachian stage and the start of the Toarcian stage of the Early Jurassic period, 183 Ma ago. At the end of the Permian, some 96% of all marine species and 70% of vertebrate terrestrial species became extinct, which makes the Permian-Triassic extinction the most severe life crisis on our Earth. The Triassic-Jurassic event resulted in the extinction of roughly half of existing species and allowed the fabled rise of the dinosaurs. The Pliensbachian-Toarcian event is associated with global extinction of many groups of invertebrates such as many of the existing ammonites at that time. What Professor Schaltegger and colleagues did was compare and combine biostratigraphy data and chemical indicators of climate and biodiversity change, such as isotopic composition of carbon and oxygen, with high precision U-Pb zircon dates from the three boundaries – Permian-Triassic, Triassic-Jurassic and Pliensbachian–Toarcian.

Recent results from the research carried out by a group of scientists including Professor Schaltegger suggest that these events were associated with rapid change from an initial cool period to hotter greenhouse conditions. They reason that this transition resulted from changing gas species emitted

during the progressive thermal erosion of Earth’s crust by plume activity. Their model is that initial gas emission was dominated by sulfur liberated from sulfide-bearing rock formations within the upper Earths’ mantle before carbon dioxide became the dominant gas. The important conclusion from the team’s analysis is that it was volcanic activity that caused massive shifts in life on Earth. Much of their reasoning is derived from the measurement of isotopes in crystals from volcanic rocks. In other words, they are explaining what happened to *life* on Earth by reconstructing the story that rocks on Earth can tell us.

**What Lies Ahead for Those Looking Back in Time?**

According to Professor Schaltegger, he was always interested in the aspect of time in geology and was fascinated by the possibility to quantify it. ‘As a second-year student I started to work in the isotope lab at University of Bern,’ he tells us. ‘Time is the basis for understanding geology, and I find fascinating that you can reconstruct and tell a story about the Earth – a Fairy Tale about the Earth.’ The group constantly tries to improve the reliability of isotopic dates. As Professor Schaltegger explains: ‘Data have to be precise, reproducible and comparable between labs and over time. Thus, coordination with laboratories at other centres is important.’ Professor Schaltegger’s group belongs to the EARTHTIME consortium, an international scientific

initiative aimed at accurately determining the history of the Earth through the integration of high-precision geochronology and quantitative study of geologic strata.

Their next steps will be to establish the possibility of extracting parts of zircon grains and analysing their chemical and isotopic composition, and thus their age, separately. This can give them greater accuracy in their determination of the age of a particular piece of zircon. ‘We need to advance our understanding of how changes in zircon and other datable minerals react to temperature, pressure, circulating fluids and other external forces,’ says Professor Schaltegger. In other words, they want to understand which processes they are actually dating - just knowing the date is only a start.

Dating geologic specimens opens the door to understanding what actually happened back there in time. Speaking geologically, for instance, give answers to questions like how and in what timescales are ore deposits formed? How are the processes deep under the Earth linked to active volcanism? How do diamond-bearing rocks come up from 150 km to 20 km depth and how long does that take? Professor Schaltegger hopes to answer these and many more questions about the Earth and its history by applying his scientific knowledge and techniques to finding out how old the hills actually are.





# Meet the researcher

**Professor Urs Schaltegger**  
Head of the Isotope Geochemistry Group  
Department of Earth Sciences  
University of Geneva  
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Professor Urs Schaltegger obtained his PhD in 1989 from the University of Berne, Switzerland. From 1989 into 1996, he held several post-doctoral research fellowships mainly funded by the Swiss National Science Foundation at various centres, including the Royal Ontario Museum's Geochronology Laboratory, the Research School of Earth Sciences at the Australian National University, the CNRS Centre for Surface Geochemistry in Strasbourg, France, and the ETH Zürich, in Zürich, Switzerland, where he held a Senior Researcher/Lecturer position from 1997–2001. In 2001, he joined the faculty at the University of Genève, where he teaches courses in mineralogy, geology and isotope geochemistry at both the undergraduate and graduate levels, as well as supervising graduate and post-graduate members of the Isotope geochemistry group.

Professor Schaltegger's research interests include developing state-of-the-art analytical techniques for high-precision Uranium-Lead dating, studying timescales for the formation of Large Igneous Provinces and their intercalibration with periods of mass extinction, calibration of geological time scale by Uranium-Lead dating of zircon found in volcanic ash beds, and timing and tempo of magmatism in the Earth's crust. He is developing concepts to understand how the mineral zircon and its Uranium-Lead isotopic system react to changes in the ambient geological environment.

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## FUNDING

Swiss National Science Foundation  
European Commission 7th Framework Programme for Research  
European Commission Horizon 2020 Programme

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## SAVING OUR DYING PLANET, WITH SCIENCE!

After just having read all about the fascinating research of Professor Urs Schaltegger and his team, one might think that mass extinctions are something of very distant past – events that occurred hundreds of millions of years ago. Unfortunately, this is not the case, as scientists are agreed that we are now right in the middle of the largest mass extinction since the Cretaceous–Paleogene event, famed for having caused the demise of the dinosaurs. Termed the Holocene extinction, or the Anthropocene extinction, this ongoing event – as you might have guessed – is almost entirely due to human activity. Although difficult to quantify, many scientists agree that we are losing species at a rate of between 1,000 and 10,000 times the normal 'background extinction rate'.

Three of the main ways that our activities have led to such a massive loss of biodiversity are through releasing greenhouse gases into the atmosphere, thus affecting the global climate, devastating the oceans through overfishing and contamination, and modifying or destroying a huge percentage of the global land area, thus replacing natural ecosystems. Other anthropogenic causes behind this extinction event include pollution, deforestation,

hunting, introduction of non-native species, and the transmission of disease.

In this section of the edition we showcase the work of several scientists, who are working to increase our understanding of how we are negatively impacting planet, and are taking steps towards undoing the damage. As climate change is the main driving force behind the massive loss of biodiversity we are witnessing, and also poses a major threat to human life on earth, we open this section with two climate science projects. First, we feature the work of Professor René Laprise at Université du Québec à Montréal, who is working to improve our climate modelling capabilities and thus, our understanding of climate change. He does this through the use of Regional Climate Modelling (RCM) – a way of increasing the accuracy of a climate simulation by reducing the area over which the model operates, thus requiring less computing power. In addition to being regarded as the father of RCM in Canada, Professor Laprise's research has influenced climate policy at the highest levels, and he has also served as a lead author of the Intergovernmental Panel on Climate Change (IPCC) 4th Assessment Report – awarded the Nobel Peace Prize





jointly with Al Gore in 2007. Also employing modelling techniques as a way of understanding anthropogenic climate change is Professor Yongkang Xue at the University of California. In our second article of this section, we highlight Dr Xue's research into the Sahel region – an area just south of the Sahara Desert spanning 5,400 km from west to east that has been plagued with 30 years of drought. His team were the first to prove that human activity is partly to blame for the mega-droughts in this region, through the use of their multi-modelling efforts. This greater understanding into how we influence our climate should help us to avoid disastrous droughts in the future, or at least mitigate their effects.

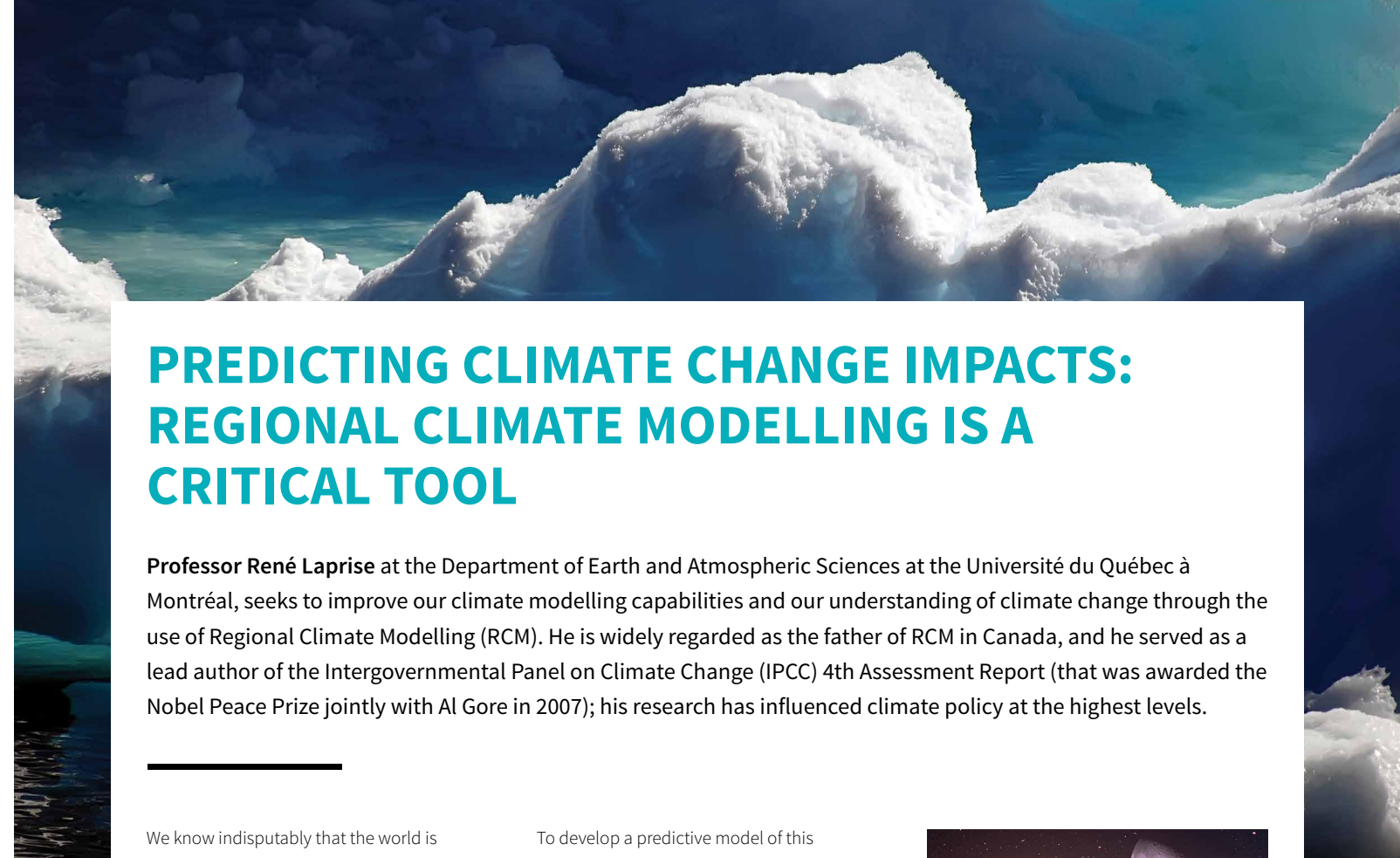
Burning fossil fuels not only leads to the release of greenhouse gases that impact our climate, it also spews toxic compounds into the atmosphere, such as nitrogen oxides, sulfur dioxide and carbon monoxide, all of which have devastating effects on human health. In our next article, we introduce the BreezoMeter team in both Haifa, Israel and San Francisco, USA, who are employing an innovative new technology to address the problems caused by air pollution. BreezoMeter is a big data platform that uses equations, statistical analysis and data collected in the field to accurately measure, forecast, and verify real time air pollution information. This information, which is now available in 7000 cities in 29 countries, can alert people to areas of high air pollution, so that they can take steps to avoid potentially damaging their health.

From air pollution, we move on to the problem of pollution in rivers, lakes and oceans. Here, we introduce Professor Theodore Endreny and his colleagues at the State University of New York, who hope to use trees to make our waterways safe for swimming, fishing and drinking. Much of the pollution in rivers and lakes results from the runoff of water from inhabited areas or agricultural land, which brings human contamination, such as sewage and chemical products, or fertiliser and

animal waste. Therefore, Professor Endreny and his team developed models to map the flow path of various pollutants, in order to identify areas where trees could be planted to filter and clean the polluted water before it reaches nearby bodies of water. They have even estimated the positive economic impacts that such a technology would have on several large cities across the globe, using a software tool they developed called i-Tree.

In keeping with the theme of water pollution, have you ever thought about how oral contraceptives might negatively impact freshwater biodiversity? This is one of the research areas that Helmut Segner and his team at University of Bern in Switzerland are focusing on. By investigating the effect of sex hormones, and in particular oestrogens, on the immune systems of fish, the team are figuring out why freshwater fish stocks might be dwindling in Europe.

Our final article of this section also deals with the sustainability of threatened fish stocks – but this time it's the Chinook Salmon, native to the Pacific Northwest. Here, we introduce the work of Dr Daniel Heath and his team of Canadian scientists, who are developing new ways to reduce the environmental impact and increase the profit associated with farming Chinook salmon. They do this by crossing domestic and wild salmon to develop high performance Chinook salmon stocks that gain weight easily on less food and have improved immune function. Farming fish with increased immune function means that they require fewer antibiotics, thus having the added benefit of decreased pollution.



## PREDICTING CLIMATE CHANGE IMPACTS: REGIONAL CLIMATE MODELLING IS A CRITICAL TOOL

**Professor René Laprise** at the Department of Earth and Atmospheric Sciences at the Université du Québec à Montréal, seeks to improve our climate modelling capabilities and our understanding of climate change through the use of Regional Climate Modelling (RCM). He is widely regarded as the father of RCM in Canada, and he served as a lead author of the Intergovernmental Panel on Climate Change (IPCC) 4th Assessment Report (that was awarded the Nobel Peace Prize jointly with Al Gore in 2007); his research has influenced climate policy at the highest levels.

We know indisputably that the world is warming. In fact, global temperatures have risen an average of 0.8°C since 1880. We have already begun to feel the cumulative effects of warming in decreasing Arctic sea ice, sea level rise, increased frost-free seasons, changes in precipitation patterns, and increased frequency and duration of severe storms.

How will these current global trends evolve in the future and how will they translate to climate impacts at the local or regional scale? How can individual regions best adapt to mitigate negative climate change impacts? Addressing these questions means understanding and predicting a climate system that is incredibly complex.

The climate system involves the atmosphere, the hydrosphere (waters on the surface of the earth, in liquid, vapour, or ice, including the oceans), and the earth's land surface. It is influenced by numerous geophysical properties such as Earth's rotation rate, the composition of the atmosphere, the depth of the oceans, land use patterns, mountains' height, and the intensity of the sun. These interacting components must be considered at various spatial and time scales. For example, cloud droplets and cloud formation occur on a fine scale of just micrometres and seconds, while El Niño Southern Oscillation (ENSO) operates on a coarse scale of several thousand kilometres and a decadal time interval.

To develop a predictive model of this massively complex system is overwhelming to think of... so how do scientists do it?

### Global Climate Models (GCM)

A Global Climate Model (GCM) is software that uses a prescribed set of inputs (also called forcings) to predict future climate. Inputs may include, for example, greenhouse gas levels or land use changes such as deforestation or reforestation activities. The IPCC (Intergovernmental Panel on Climate Change) describes GCMs as 'the most advanced tools currently available for simulating the response of the global climate system to increasing greenhouse gas concentrations'.

GCMs are computer programs based upon numerical models that take the geophysical properties that affect earth's climate and use governing equations — known laws of physics — to determine exchanges of momentum, energy and mass between the atmosphere, hydrosphere and land surface. GCMs can be used to calculate changes over time for many variables, for example: pressure, humidity, precipitation, ocean salinity, sea ice, or snow cover, etc.

### It's a trade-off: model resolution versus computing power

GCMs apply a three-dimensional grid to the earth's atmosphere and oceans. At each



grid point, the supercomputer running the model solves the governing equations to provide data on the climate variables being simulated. The typical GCM consists of 30–60 vertical layers along its vertical axis and grid points 200 km apart on its horizontal axis in the atmosphere. In total, a GCM may consist of over a million of grid points across the full globe.

In addition to the spatial resolution, the temporal resolution (time-step) of GCMs also must be considered. A GCM with a temporal resolution of 30 minutes requires almost two million time-steps in a 100-year climate simulation. At each time-step, all model variables are calculated at each of the (millions of) grid points!



**‘Given the quasi impossibility (and undesirability!) of experimenting with the real climate, models serve as a computational laboratory in which one can carry experiments to test hypotheses’**



Increasing the resolution of a model involves either increasing computer processing speed or increasing the time it takes for completing a simulation. As Professor Laprise explains: ‘if a simulation took one week of computer time with a 200 km grid, it would take over two years of computations with a finer scale grid of 40 km – too long a waiting time for most!’ The average grid size of GCMs participating in century-long climate projections for the IPCC 5th Assessment Report was about 300 km. For perspective, at this scale, the UK would be covered by only about a half a dozen grid cells.

To keep within the bounds of current computing capabilities, and to reduce costs, climate modellers are forced to take some shortcuts. These include simplifying some fine-scale processes – for example, cumulonimbus cloud formation in thunderstorms, which, at an average of

about 10 km in size, cannot be represented in a model with a resolution of 200–300 km. Although these fine-scale processes cannot be resolved by the model, they have an impact on the global climate and need to be accounted for, so scientists use parameterisation processes — mathematical equations that generalise the influence of these fine-scale processes on large-scale conditions in the model. As you might expect, the use of parameterisation processes can introduce substantial uncertainties into the model.

Computing advancements have enabled substantial increases in GCM resolution since the 1990s; but, the relatively coarse scale of GCMs continues to be a roadblock for climate modellers. Increases in the resolution of climate models are vital for predicting regional climate change impacts and for designing effective adaptation strategies for

those consequences we can’t mitigate (for example, water resource management, or flood and coastal erosion prevention). The solution to this conflict between computing costs and the need for higher resolution data may lie in the use of Regional Climate Modelling.

**Regional Climate Modelling**

Regional Climate Modelling (RCM) has emerged over the past 20 years and allows researchers to remain within the bounds of affordable computing power while achieving, as Professor Laprise describes it, an ‘unprecedented amount of detail’ in climate simulations over sub-regions of the globe.

Reducing the area over which the model operates decreases computing cost and allows increasing spatial resolution. For example, EURO-CORDEX (the European arm of CORDEX, an international effort to produce improved regional climate change projections for all land regions worldwide) is working on grid cells of 12 km and making projections up to the year 2100. RCMs with grid sizes of 2.5–4 km are also being tested and models of this scale are able to reduce the simplification of convection, cloud and precipitation processes that can produce modelling uncertainty but is necessary in coarse-scale GCMs.

To make climate projections an RCM receives data from a GCM at its lateral boundaries (edges). In this way, the GCM provides starting inputs and driving information over time on large-scale variables (such as atmospheric temperature, winds and humidity), while the RCM provides increased resolution to calculate more precisely physical processes with improved representation of geophysical features within the study region – for example, mountain ranges and land use patterns. As a result, RCMs can inherit biases of the GCM that supplies data at its boundaries – for example, if the GCM is modelling a large-scale feature (such as El Niño – Southern Oscillation (ENSO)) poorly, then the RCM will inherit these biases and provide a poor simulation of regional ENSO effects. However, in cases where GCMs fail primarily due to coarse resolution of regional geophysical features and physical processes, RCMs are extremely helpful. For example, RCMs can be used to describe regional weather features such as lake-effect snow belts, regional monsoons, or the detailed precipitation patterns in regions with complex, fine-scale mountain ranges.



**‘After working on the development of the first generation of Canadian Global Climate Model at the Meteorological Service of Canada, it seemed natural for me to move on to work on developing a high-definition (fine-mesh) Regional Climate Model that would allow resolving weather sequences with unprecedented amount of details’**

**Model Intercomparison Projects increase model validity**

How are model results tested and validated? Professor Laprise says that, ‘while the main goal of climate models is to perform future climate-change projections, most of the efforts of climate modellers is dedicated to performing hindcast simulations for the recent-past climate and comparing these with available observational data to evaluate the skill (quality) of models.’

Because of the chaotic and complex nature of earth’s climate system, to identify statistically significant trends in climate modelling requires the use of model intercomparison projects, in which simulations from several models are pooled in order to produce mean values that

outperform the ability of any single model.

An example of a model intercomparison project is CORDEX (COordinated Regional climate Downscaling EXperiment), an international coordinated effort to produce high-resolution climate change information derived from multiple models and suitable for impact and adaptation work. Professor Laprise says CORDEX and other model intercomparison projects are ‘most helpful to evaluate the skill of models and identify weaknesses that call for attention’.

**Future research and influences on policy**

Professor Laprise describes his current research as ‘strongly focussed on methodological aspects of Regional Climate Modelling’, including identifying the most optimal conditions for the application of RCMs with respect both to the size and the location of the study area, as well as the associated impact of the resolution jump from GCM to RCM at the edges of the RCM grid. His work aims to provide a set of operational rules that scientists can follow to achieve optimal application of RCMs and to reduce the uncertainties associated with the use of RCMs.

He and his colleagues are working to improve and validate several aspects of the fifth generation of the Canadian Regional Climate Model (CRCM5) and are also working on coupling the CRCM5 model with a regional ocean model such as NEMO (Nucleus for European Modelling of the Ocean).

In parallel to model improvement and applications, it is important to use detailed diagnostics to interpret the simulations and compare them to analyses of observations. With his team of students and research associates, Professor Laprise has developed recently a detailed energy budget formulation that allows evaluating the various energy transformations taking place in weather storms, and how these will change in a warmer climate.

Professor Laprise is one of the many scientists who volunteer their time and expertise as Lead Authors on assessment reports produced by the Intergovernmental Panel on Climate Change (IPCC). The IPCC is the international body for assessing the science related to climate change. IPCC assessments provide a scientific basis for governments at all levels to develop climate related policies, and they underlie negotiations at the UN Climate Conference – the United Nations Framework Convention on Climate Change (UNFCCC).

IPCC describes its assessments as ‘policy-relevant but not policy-prescriptive’. The assessments present projections of future climate change based on different scenarios related to land-use change, population growth, energy use, etc., and discuss the risks that climate change poses as well as the social and environmental implications of different response options, but they do not tell policymakers what actions to take.

In 2007, the IPCC and former U.S. Vice-President, Al Gore, were jointly awarded the Nobel Peace Prize ‘for their efforts to build up and disseminate greater knowledge about man-made climate change, and to lay the foundations for the measures that are needed to counteract such change’.





## Meet the researcher

### Professor René Laprise

Professor, Department of Earth and Atmospheric Sciences  
Chair, Graduate Study programmes in Atmospheric Sciences  
Member, ESCER Centre for the Study of Simulation of Climate at Regional Scale  
Université du Québec à Montréal (UQAM)  
Canada

Professor René Laprise is widely recognised as the father of Regional Climate Modelling (RCM) in Canada. He has been a member of the faculty at Université du Québec à Montréal (UQAM), Montréal, Québec since 1988, and within the department of Earth and Atmospheric Sciences since 1995. Among his many accomplishments, is Professor Laprise's contributions to the Intergovernmental Panel on Climate Change (IPCC) 4th Assessment Report (AR4) that was awarded the Nobel Peace Prize in 2007, ex aequo with former vice-president of USA, Al Gore. He served as Principal Investigator of the Canadian Network for Regional Climate Modelling for 15 years, and has received numerous honours for his contributions to the field. He has published over 129 peer-reviewed journal articles and 5 book chapters, and has trained 85 graduate students and 14 postdoctoral fellows during his tenure at UQAM.

#### CONTACT

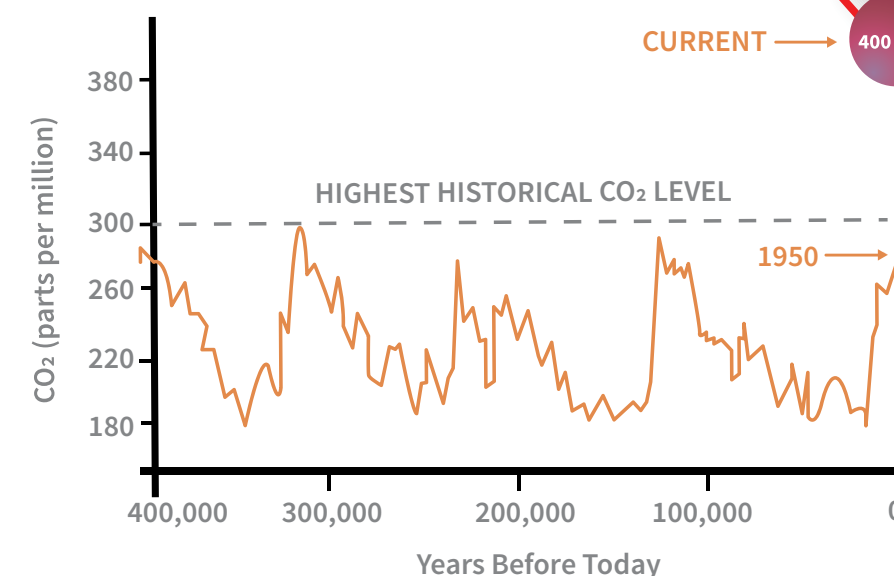
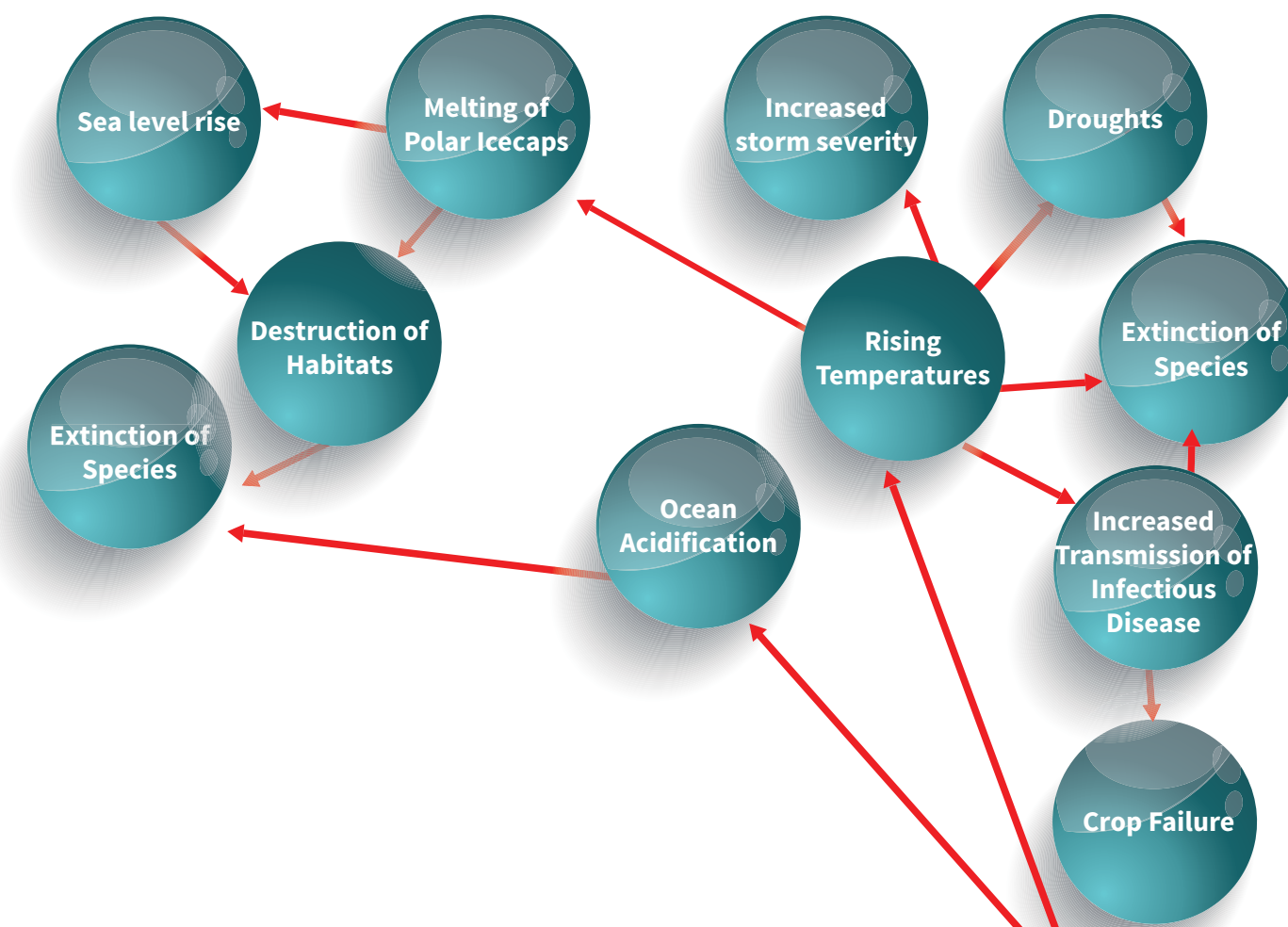
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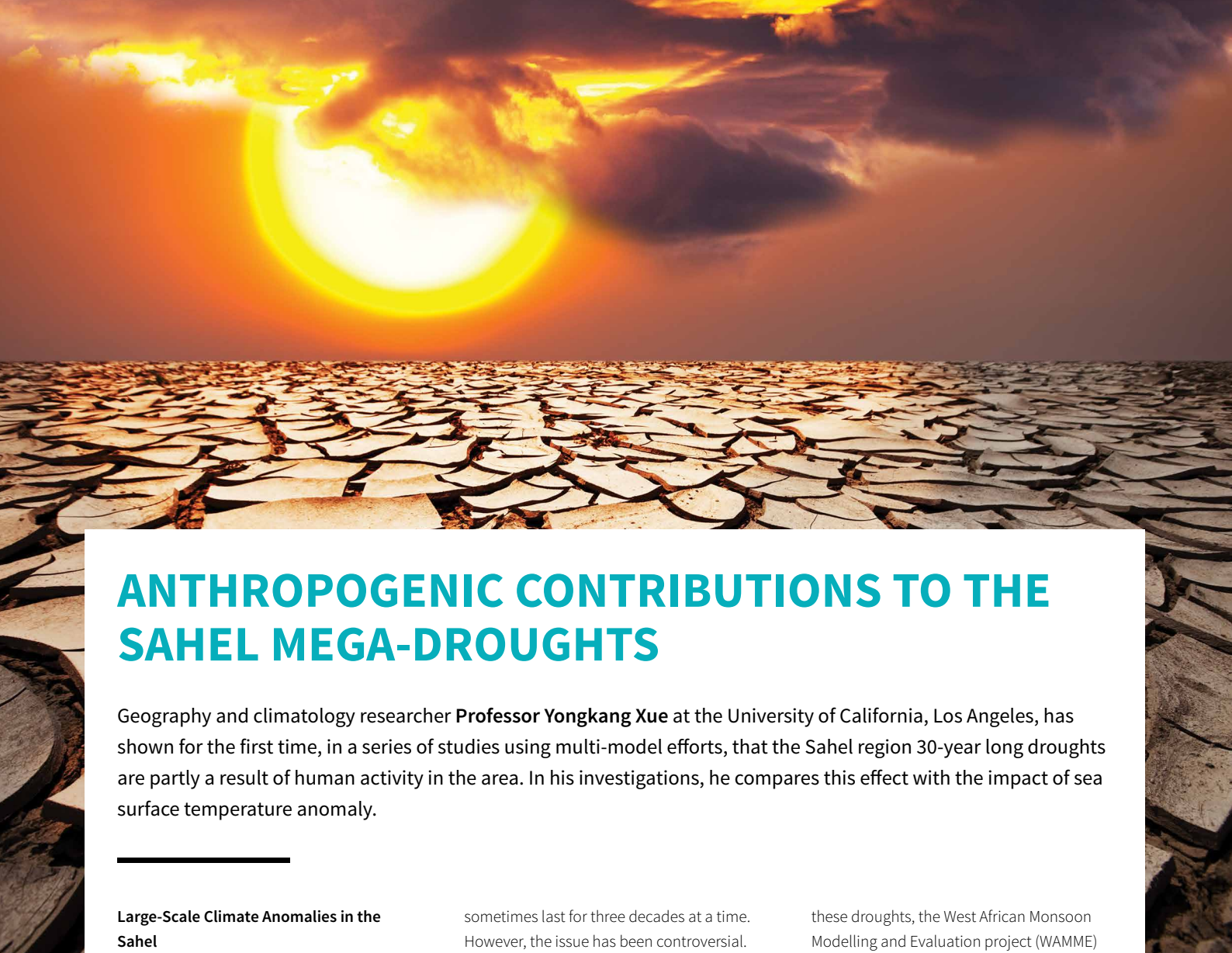
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## EFFECTS OF ANTHROPOGENIC CLIMATE CHANGE







## ANTHROPOGENIC CONTRIBUTIONS TO THE SAHEL MEGA-DROUGHTS

Geography and climatology researcher **Professor Yongkang Xue** at the University of California, Los Angeles, has shown for the first time, in a series of studies using multi-model efforts, that the Sahel region 30-year long droughts are partly a result of human activity in the area. In his investigations, he compares this effect with the impact of sea surface temperature anomaly.

### Large-Scale Climate Anomalies in the Sahel

The Sahel is one of the most extraordinary, striking regions on the planet. Home to a population of 50 million people, the Sahel forms a sub-Saharan belt with a maximum width of 1,000 km, spanning the entire 5,400 km of the African continent from the Atlantic Ocean and all the way to the Red Sea. Over the past 50 years, the population in this area has doubled and there are signs that it may double again by 2050. This region covers parts of Senegal, Mauritania, Mali, Burkina Faso, Algeria, Niger, Nigeria, Chad, Sudan, South Sudan, Eritrea, Cameroon, the Central African Republic and Ethiopia. Aside from being a massive geographical feature, the Sahel represents the ecological, climatic, and biological transition area between the Sahara Desert in the north and the Sudanian Savanna in the south. Unfortunately, this area is prone to serious droughts. 'West Africa or Sahel had the most severe droughts on Earth during the 20th century,' Professor Xue tells us. Climatologists have taken into consideration the effects of anthropogenic land use and land coverage change (LULCC) as a factor for these droughts known to

sometimes last for three decades at a time. However, the issue has been controversial.

The Sahel saw several severe drought periods during the 20th century in the 1940s, 1960s, 1970s and 1980s, all of which were followed by famines that claimed human lives and livestock, and lead to serious land degradation. However, the most lengthy and intense decades of drought were those between the 70s and 80s. 'The Sahel drought lasted from the 1970s to the 1980s, which was the longest and most severe large scale drought in the world in the last century,' Professor Xue explains. 'The United Nations had two international desertification conferences due to this drought. The causes of the drought were under intense debate, and some claimed it was natural and mainly due to sea surface temperature variability. Other scientists, such as myself, consider that humans, through land use and land cover change, were also one of main causes. Exploring the human effect on this issue is quite challenging. I believe my research is important to prevent such disastrous droughts and mitigate their effect.'

In an international effort to investigate

these droughts, the West African Monsoon Modelling and Evaluation project (WAMME) was initiated as part of a climate science collaboration called the Coordinated Enhanced Observing Period (CEOP)/the Global Energy and Water cycle Exchanges (GEWEX) investigating climate variability and anomalies. The GEWEX uses satellites, observational data, and continental-scale experimental models to create better climate predictions and study monsoon systems. Monsoons are seasonal wind direction reversals and precipitation changes that characterise Asian-Australian and West African regions, such as the Sahel. The WEMME collaboration aims to better understand the mechanisms behind the Sahel drought that impacts water availability in monsoon regions. The collaboration also hopes to uncover the role of oceanic, land, and atmospheric forcing factors in the West African climate system. Within the collaboration, WAMME and WAMME II employ state-of-the-art earth system models to explore how ocean, terrestrial ecosystem, and aerosol processes and their interactions influence the weather and climate variations over decades in West Africa.

**‘My work focuses on how land-atmospheric interactions affect the weather and climate – especially the effect of human-induced land use and land cover changes’**



### A Crash Course in Climate Science

During the first WAMME and the second (WAMME II) projects, Professor Xue and his colleagues developed new mathematical tools and experimental models to understand droughts. 'My work focuses on how land-atmospheric interactions affect the weather and climate – especially the effect of human-induced land use and land cover changes,' he explains. 'To reach this goal, I have developed comprehensive land models that can realistically represent the land processes (including surface water, carbon, energy and ecosystem processes) and their interactions with atmosphere. Using these models, I explore how land degradation affects the West Africa and East Asian droughts, how land surface processes contribute to the weather and climate processes, including the atmospheric circulation, and how spring land surface anomalies in the western mountain area in the northern hemisphere affect the downstream droughts and floods.'

From a geographical coverage perspective, climate evolution models are either

atmospheric general circulation models (AGCM) or regional circulation models (RCM). In both cases, the climate model requires the studied area to be divided into small sub-regions by means of an orthogonal grid. Although at the large scale the simulated climate evolution may not influenced by the size of the grid, predictions for small regions best work when the grid resolution is high and the size of each grid square is small. The problem posed by trying to make small-scale predictions in global models is that high resolutions are computationally intensive and therefore require massive computational resources. Fortunately, current computer technology is able to satisfy this demand. The models need to take into consideration mathematical and physical processes that represent the dynamics of the climate and forcing agents.

The attribution of climate fluctuations to specific forcing agents such as sea surface temperature, land use or aerosols is one of the central subjects for climate science. A forcing agent can be defined as any factor capable of influencing a mathematical model composed of several differential

forcing functions, such as solar radiation, greenhouse gases, etc. The LULCC and sea surface temperature are also represented in climate models by means of forcing functions. An imposed forcing function represents the unyielding influence of a single agent on the system over the studied period of time. In short, climate models with forcing functions simulate the action of an imposed factor on the dynamical system of equations; whereas temperature, humidity, rainfall and wind can change due to this function.

In order to systematically understand climate behaviour and trace it back to the factors causing it, climate scientists have a range of mathematical methods at their disposal. For instance, they can use statistics to compare the observed climate variability with respect to time with the responses of a model to the imposed forcing. If the variations of the actual climate match those of the model response and vice versa, it follows that this forcing plays a role in climate variability and anomalies. To create proper models for such experiments, scientists must first verify the behaviours, such as how well the models





generate a reasonable representation of a West African monsoon through climate model experiments, as did in the first WAMME project. If models have deficiencies in producing some basic climate behaviour, such climate models can be tweaked by improving mathematical and physical processes that they represent in successive iterations until a good model behaviour is established.

**The Effect of Land Use and Land Cover Change**

Knowing that population activity and dynamics in the Sahel region trigger a large scale effect capable of altering the regional climate over decades, Professor Xue and his collaborators in the WAMME project tested their working hypothesis by employing several climate models from different institutions across the world. These models have included forcing functions corresponding to the action of aerosols, sea surface temperature, and LULCC.

WAMME II involved working with eight global circulation models and four regional climate models with spatial resolutions ranging between 20 and 200 km. The models included comprehensive aerosol schemes and biophysical representations of processes taking place on the surface of the land. To test the impacts of sea surface temperature on the rainfall anomaly, the WAMME II imposed the maximum sea surface temperature anomaly observed in the 1950s – a period with abundant rainfall – in one experiment and the maximum observed sea surface temperature in the 1980s – a period with severe drought – in another experiment. The difference in simulated rainfall between these two experiments was compared with the observed difference between the 1950s and the 1980s. The analysis considered the impact of sea surface temperature anomalies in different ocean basins, such as the Pacific Ocean, because the effect in Sahel can be a manifestation of direct forcing from the neighbouring ocean, or indirect forcing, in which remote oceans can interact with the region via atmospheric anomalies.

To test the LULCC effect, the procedure used a control simulation, where normal (or a natural without human intervention) land cover conditions were imposed as forcing, such as the 1950s land conditions. In another LULCC experiment, land use and land cover maps in the 1980s were imposed. Comparisons between the results of the control simulation and the LULCC experiment highlight the effect of LULCC on the 1980s drought.

The LULCC experiments were based on maps of crop and pasture

**‘I believe my research is important to prevent such disastrous droughts and mitigate their effect’**

surfaces and the variations of these surfaces during the past centuries. The land usage map clearly showed a pattern of significant change in land use over the Sahel in the past century, which largely levelled off after the 80s. The LULCC effect map is characterised by areas where natural vegetation degraded. Introducing LULLC as a forcing factor resulted in more than a 30% decrease in broadleaf trees and roughly 15% decrease in grasses. Consequently, the local albedo increased by up to 0.10 from a value of ~0.16 in some areas. The maximum impact occurred in the degraded land area, and the strongest climatological anomaly appeared in West Africa.

As planned from inception, the models have evaluated the sensitivity of the Sahel climate LULCC and compared the effect with that caused by sea surface temperature. Although sea surface temperature forcing explained a large part of the rainfall deficit, the LULCC accounted for a wet signal to the south of Sahel – a feature of the rainfall anomalies observed in the 80s Sahel drought – which cannot result from sea surface temperature forcing. When imposing the sea surface temperature forcing to the maximum feasible values as discussed earlier, Professor Xue and his collaborators found that, with maximum possible SST forcing, SST can account for up to 60% of the precipitation anomaly between the 1950s and 1980s, whereas LULCC, which is probably also a maximum possible LULCC scenario, accounted for 43%. This is less than the surface temperature effect but is still a significant proportion, and should be considered as a first order effect. A comparison of the anomalies caused by the forcing due to sea surface temperature and LULCC revealed that SST causes a precipitation anomaly to form near the equator, starting in late winter and early spring, whereas the LULLC-induced anomaly only develops when a monsoon rainfall band moves into the Sahel region where the forcing is applied. The maximum response to LULCC forcing occurred during the summer monsoon months. This phenomenon shows that SST has a role in triggering the Sahel drought but land surface processes respond to the drought and amplify it.



# Meet the researcher

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Professor Yongkang Xue received his Ph.D. in meteorology from the University of Utah in 1987. He is a fellow of the American Meteorological Society and has multiple research interests such as climatology, atmosphere-land surface modeling and dynamics, regional dynamic downscaling, meteorological prediction, and remote sensing. His work focuses on studying how land-atmospheric interactions affect the weather and climate, especially the effect of human induced land use land cover changes. For this purpose, he developed realistic land models which take into consideration all land processes, such as surface water, carbon, energy, and ecosystem processes, and their interactions with the atmosphere. Currently, the areas where he focuses his research include West Africa, Continental U.S., East Asia and South America, Throughout his career, Professor Xue has published 183 research articles which have been cited about 8,000 times.

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**FUNDING**

NSF

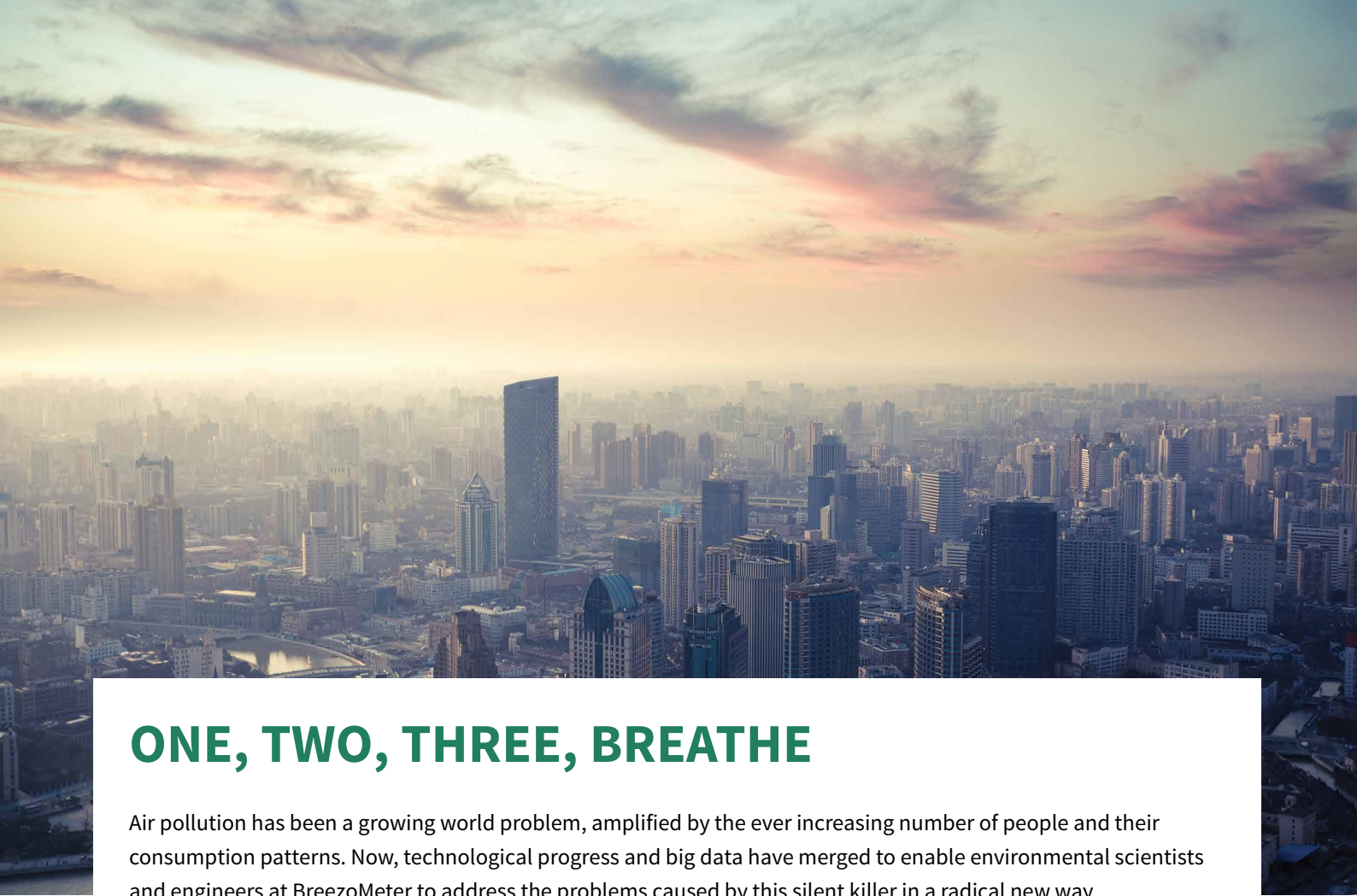
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# ONE, TWO, THREE, BREATHE

Air pollution has been a growing world problem, amplified by the ever increasing number of people and their consumption patterns. Now, technological progress and big data have merged to enable environmental scientists and engineers at BreezoMeter to address the problems caused by this silent killer in a radical new way.

## Battling Urban Air Pollution

A person can survive three weeks without food, three to five days without water, and only a few minutes without air. Despite the fact that each of us breathes almost 11,500 litres – or approximately 3,000 gallons – of air every day, it is often treated as less important than other resources. Yet ambient air pollution can trigger many illnesses and significantly shorten the life span of individuals, even in the absence of a fatal disease.

In 2012, air pollution caused 3.7 million deaths, corresponding to 6.7% of the planet's population and in 2015 that number climbed to 5.5 million people. In fact, ambient air pollution accounts for more than 20% of ischaemic stroke and heart disease cases, roughly 16% of lung cancer cases, about 13% of respiratory infection deaths, and an estimated 11% of chronic obstructive pulmonary disease (COPD) – a lingering disease decreasing the sufferers' quality of life and known to have caused the death of the celebrated actor Leonard Nimoy. On a daily basis 92% of all people inhabiting urban

areas are exposed to levels of air pollution higher than those recommended by the World Health Organisation. Although airborne particles and noxious gases decrease the air quality worldwide, the problem is disproportionately larger in poorer countries. However, pollution levels continue to rise even in developed countries. But what if technology would enable us to forecast pollution just like we can forecast the weather? Smart cities are currently under development, while smart buildings are already a part of our daily lives. So what if this infrastructure could be used so that people worldwide would receive high air pollution alerts in order to avoid areas of foul air?

With these problems in mind, a group of scientists and engineers at BreezoMeter started to search for a solution that would enable people to decrease their total exposure to polluted air. Making predictions and issuing actionable alerts is not easy because air quality in the same region changes several times a day and is governed by complex dynamics. Therefore, the team at BreezoMeter realised that this objective is not impossible. Knowing in advance what

is to come, urban inhabitants could plan better alternative routes to or from work and home, use indoor air purifiers, and even move their regular jogging route to a safer location. At the same time, alerts from a main computer framework could be accompanied by suggestions for smart city dwellers regarding the best course of action when a pollution wave moves in. Additionally, several industries could work towards creating better air and a better standard of life by incorporating the knowledge regarding air pollution in their products. For example, automotive manufacturers could create better air filters, and heating and cooling installations could be better equipped to handle the rising air pollution levels. In this vein, a smart home could activate supplementary filters upon receiving pollution wave alerts, whereas real estate brokers would be able to provide their clients with detailed heat maps showing the safest and healthiest places to live. To incorporate all these functions and more, Dr Benmoshe in collaboration with a handful of researchers and engineers at BreezoMeter develop a proprietary algorithm capable of offering never before seen accuracy and certainty.

## Forecasting Air Quality

Similar to climate and weather forecasting models, BreezoMeter is a big data platform that uses physical equations, statistical analysis and data collected in the field to predict and track the evolution of air pollution waves through inhabited areas. 'BreezoMeter maps the world's air pollution, delivering dynamic, real time, and up-to-city-block-level air quality data worldwide,' Ziv Lautman, BreezoMeter's Co-founder and Chief Marketing Officer explains. 'Our mission is to help cities and businesses improve the health and quality of life of millions of people worldwide, by providing the most accurate air quality data in a format as simple, intuitive, and actionable as weather data. BreezoMeter's big data analytics determine the dispersion and flow of air pollution from a combination of layered data sets derived from governmental sensors, satellites, weather patterns, transportation dynamics, and other environmental sources. Tied together with proprietary algorithms, this combination provides users with accurate air quality data that is up to the city block.'

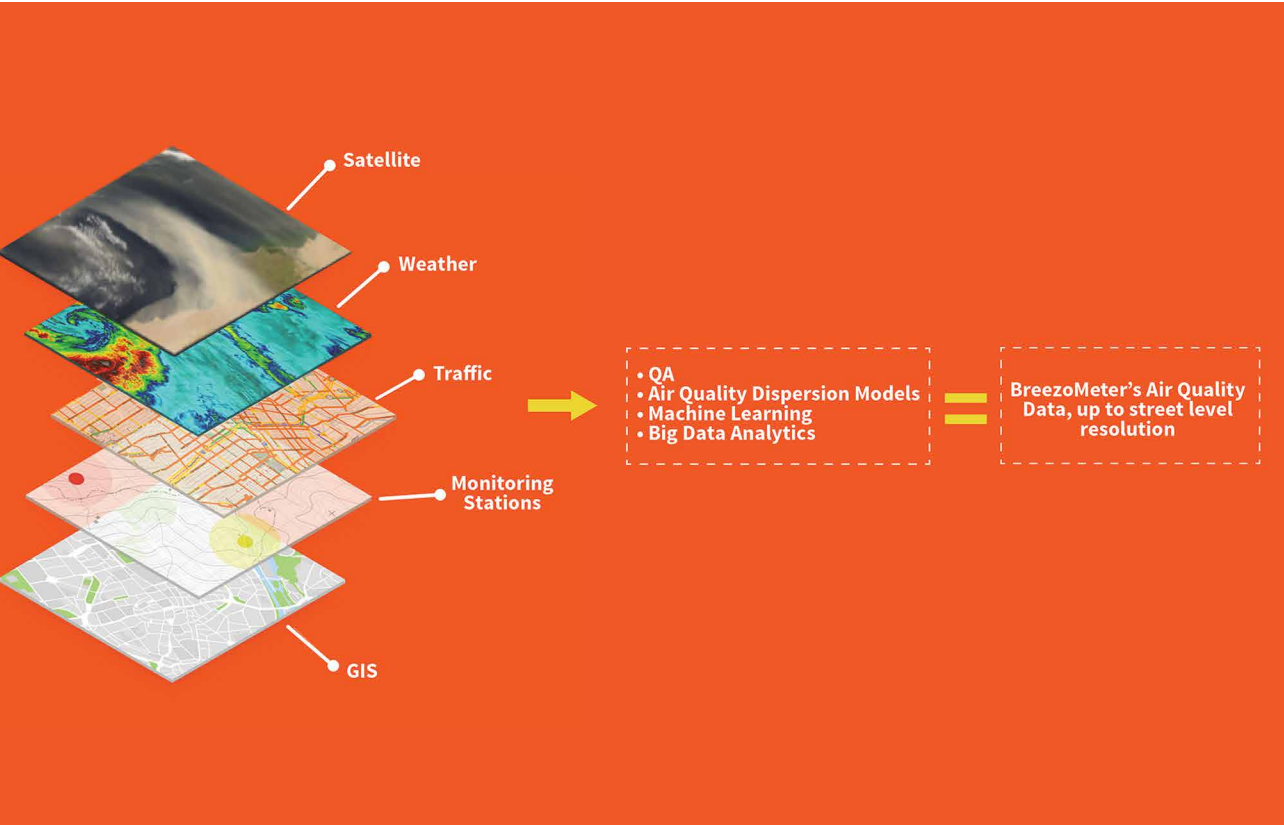
Dr Benmoshe together with the algorithm team at BreezoMeter were in charge of finding a way to accurately measure, forecast, and verify real time air pollution information. This is a challenge for several reasons.

One of these reasons is the provenance of data incorporated in the analysis. Whereas other applications for air quality analysis take data from unverified sources, such as smartphones, individual reports, and off the ground report sources such as satellites, the researchers at BreezoMeter needed to find a way to also verify the data in real time and make sure the information is completely reliable. Another problem is that atmospheric circulation requires extreme computational power for accurate predictions, especially in models with high spatial resolution such as those currently incorporated in the platform.

The answer to the problem of forecasting the wave motion of pollution is inspired by global and regional weather models, which are used by meteorologists to track the progress of weather and make predictions. The background BreezoMeter's research and development team has in atmospheric science, turbulent flow, and convection, helped BreezoMeter use the scientific principles and mathematical modelling tools with the decision on how the algorithm should behave for optimal results. These models divide the studied map or space in a grid, whose size is equal to the resolution of the model. In the BreezoMeter's case, the calculations use a grid resolution finer than 500 metres – the size of a block. As Dr Benmoshe points out: 'we are the first

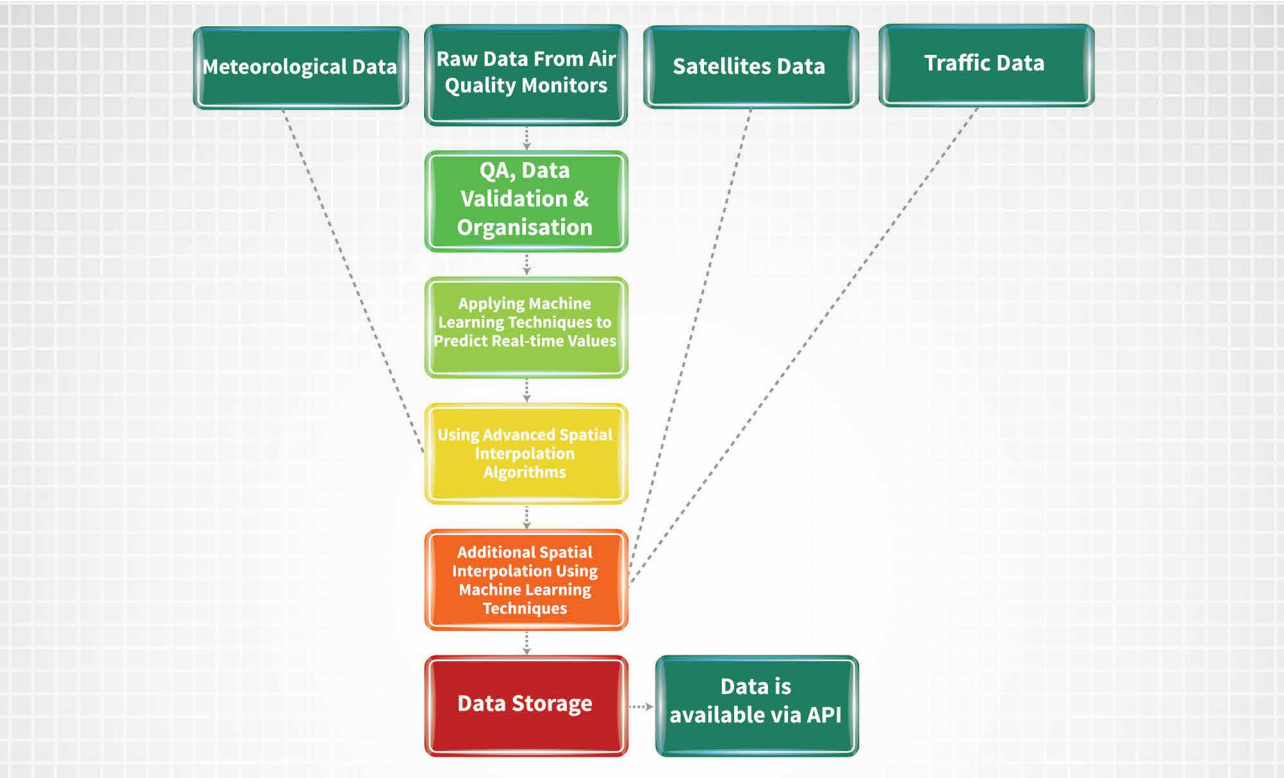
company in the world to provide spatially interpolated data at the resolution level of a city block.'

In its final form, the algorithm uses data from many sources to determine air quality levels. The main sources are governmental monitoring stations, which feed the platform with hourly concentration readings for air pollution. At the same time, supplementary data from satellite measurements, meteorological and traffic data, and data regarding types of land cover are added to increase the accuracy of the prediction, together with air quality models such as the European programme Copernicus Atmosphere Monitoring Service (CAMS). Air quality models track changes in pollution levels due to dispersion and chemical reactions; the CAMS offers no less than seven circulation models, which are validated every three months. Additionally, insufficient data will never be a problem for the platform, as Dr Benmoshe explains: 'Since we validate our data on every spot of the grid, we can know in real time if our algorithm's results are not accurate enough. We want to make sure we provide the most accurate data, so in cases of extremely thin coverage or inaccurate input data we might decide not to produce a map for a specific area.'





‘Our story began in 2012 when we were looking for pollution-free areas to settle in and buy a house – our families have health sensitivities, including asthma. As environmental engineers, we are too much aware of the correlation between environment and health, and refused to compromise on air quality’



Precision and Dynamics in Real Time

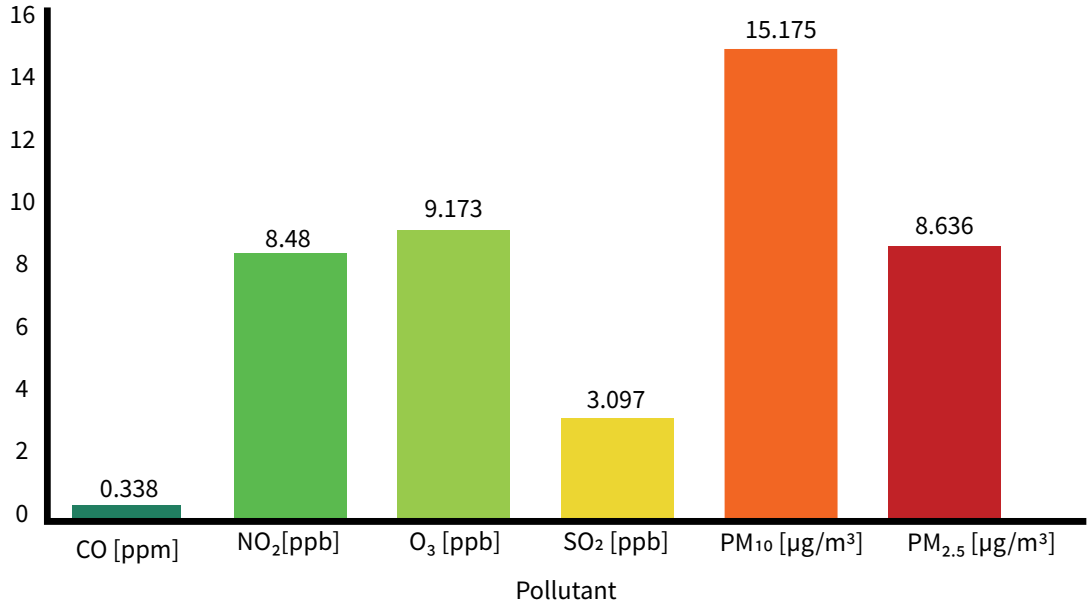
Although important for country- and city-level air quality planning, public communication, and regulatory compliance, ground-level measurements alone are not sufficient to provide global coverage to estimate exposure because of the spatial biases in the availability of ground level measurements, the differences in measurement approaches among jurisdictions, and the absence of details about measurement data in some instances. Ground-level measurements of air pollution, particularly PM2.5, are unavailable in much of the world, and especially in many of the low- and middle-income countries. In addition to the inadequate and highly uneven coverage of ground-level measurements, measurement protocols and techniques are not standardised globally, with different quality control programs and different numbers of samples to arrive at annual averages. Even for measurements made by (similar) filter-based approaches, filters are equilibrated at different relative humidity conditions prior to weighing (for example, 35%, 40% and 50% relative humidity in the United States, Canada and European Union, respectively) and therefore

are not completely equivalent. In addition, PM10 measurements and PM2.5/PM10 ratios are commonly used to infer PM2.5 concentrations for ground-level estimates. Therefore, surface measurements, although a key component of any global assessment approach, cannot be used solely to derive global exposure estimates.

Satellite-based measurements can help provide estimates for areas with no ground-level monitoring networks. But even in North America, where monitor density is high in populated areas, studies have indicated that satellite-based estimates do provide additional useful information on spatial and temporal patterns of air pollution (Kloog et al. 2011, 2013; Lee et al. 2012). Furthermore, in a large population-based study in Canada the magnitudes of estimated mortality effects of PM2.5 derived from ground measurements and satellite-based estimates were identical (Crouse et al. 2012). Satellite-based estimates nonetheless must be used cautiously, as discussed shortly (World Bank and Institute for Health Metrics and Evaluation. 2016. The Cost of Air Pollution: Strengthening the Economic Case for Action. Washington, DC: World Bank).

To make sure the raw data is reliable, the team constantly monitors its behaviour. Each new registered value is tested by several methods to confirm it falls within the normal trend and range, and is not a false data point. In fact, the data is normalised by eliminating invalid outliers, which may arise from sources such as uncalibrated or faulty monitoring stations. The platform uses the values measured at stations to interpolate the values between the stations, and then cross verifies it against the layer of data from other sources, at the same time taking meteorological aspects into account. Because the results are calculated in advance for billions of points worldwide, they are available for users instantaneously. Naturally, this process generates big data, and according to Emil Fisher, BreezoMeter's Co-founder and Chief Technology Officer: 'every hour we process more than 680 GB of data while calculating the air quality for more than 271 Million grid points worldwide. In this process we produce new 29 GB of data every hour. In order to succeed in this mission, we use Google Cloud services to manage our data, and run hundreds of CPUs every second. Currently, we store a total amount of more than 300,000 GB of data in the cloud.'

Root mean squared error (RMSE)



Results of 5000 random 'leave-one-out' tests, performed with data from June and July 2016. 100 hours of data were chosen randomly, for each of them 50 monitoring stations were chosen randomly. For each of the time-station pairs we calculated our algorithm's result at the station coordinates, then compared the result with the measurement from the relevant hour. The RMSE results are satisfactory.

Accuracy Above All

The algorithm's accuracy is regularly validated by several statistical methods. For instance, the leave-one-out cross validation method compares a prediction map with a real set of data. By eliminating the real input data set from the calculation and letting it make an interpolation to find the prediction, scientists can verify the accuracy of the algorithm by comparing the result at the end with data from the field. These tests are being run thousands of times, so that the team can understand whether they predict the right correlations. And above all that, BreezoMeter's algorithm is one of the fewest currently capable of analysing pollution dispersion in real time, which means that the data is dynamic, relevant and based on the location of the individual using the platform – not just the location of the pollution monitoring stations, like other

applications. As stated before, air pollution changes several times a day and is governed by complex flow dynamics. Therefore, in order to provide individuals accurate air quality levels, pollution data must be analysed as BreezoMeter does. Since the algorithm incorporates machine learning and is capable to adapt based on previous experience, the platform will function in real time even if the source data is not available in real time.

Although several other air quality monitoring applications exist, a major challenge for users is that reading scales are not standardised between continents, or even between countries. To address this issue, the BreezoMeter team created a standard Air Quality Index scale ranging from 0 (worst) to 100 (best) and divided it into five levels, each of which is associated with health recommendations for the general population

and at-risk groups such as children and cardiovascular patients. There are six main pollutants tracked by BreezoMeter, namely carbon monoxide, nitrogen dioxide, ozone, sulphur dioxide, and two size thresholds of particulate matter – with diameters below 10 and 2.5 micrometres. Given its ability to track particulate matter, BreezoMeter can also show pollution due to fires.

At this point, BreezoMeter's air quality data is available in more than 7000 cities in 29 countries, and the goal is to map the entire world by 2017. When asked about their future plans, Ran Korber, BreezoMeter's Co-founder and Chief Executive Officer tells us: 'We are constantly improving our algorithms, adding data layers and aiming to a higher resolution of data. This is our main area of focus as our mission it to provide the most accurate air quality data'

About BreezoMeter

With over 50M daily users in 29 countries, BreezoMeter is the world's leading real-time air quality analytics provider. The company offers its air quality data as a service via a simple API integration to various products and services. BreezoMeter also offers a set of advanced solutions for Smart Cities such as: Air Quality Command and Control Platform, SMS-Sensor Management System and Optimization, Data analysis and more.

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# ERADICATING WATER POLLUTION ACROSS THE GLOBE – WITH TREES

Environmental engineer **Professor Theodore Endreny** and his colleagues at the College of Environmental Science & Forestry of the State University of New York want to improve the world by devising ways to make our waterways safe for swimming, fishing and drinking, using trees!

## How Much of the World's Water Can We Actually Use?

As every school child learns, three-quarters of the Earth is covered with water, but 96.5% of it is held in our oceans. We can swim in it and fish in it, but we can't drink it – it's salt water. Even so, some of it is too polluted even to swim or fish in. The freshwater we need to drink – only 3.5% of the Earth's total water – is largely tied up in glaciers, the polar ice caps and permanent snow, as well as the water vapour in the atmosphere, ground water and soil water that are not easily accessible. What we're left with is the water in freshwater rivers and lakes, a measly 0.00001% of the Earth's water. But the Earth's animals and plants contain about a quarter of this amount, and as we use that water to live it must be replenished. If water we suck out of the lakes and rivers isn't replaced without pollution, we have no place to get our water. Clearly humans – and all animal and plant life – are intimately connected to the Earth's water supply for life itself. It is this precious, life-sustaining commodity that Professor Theodore Endreny and his colleagues in Environmental Resources Engineering want to protect and preserve. And to do that, they talk about the trees.

## Keeping the Water Clean by Using Trees as Living Filters

It is estimated that half of the world's hospital beds are occupied by patients suffering from disease attributable to contaminated or polluted water. In poor countries, unsafe drinking water leads to diarrhoeal diseases, such as cholera. The World Health Organization estimates that 88% of cases of diarrhoea globally result from unsafe water supplies, while the United Nations estimates that 10% of the world's disease burden could be reduced with improvements in water quality, such as through sanitation, hygiene and management of critical water resources. This is clearly an important and global problem, the costs of which can reach into the trillions of dollars. And besides the health implications, the economic issues related to polluted and limited water supplies cost money, too. It is estimated that in the United States alone that water pollution by nitrogen and phosphorus costs the government, water purification facilities and individuals at least \$4.3 billion USD annually. The social, economic, and environmental impacts of water pollution are huge. So how do our water supplies get so polluted? According to Professor Endreny: 'non-point source runoff of nutrients from agriculture and urban areas

has impaired waterways, keeping them from being swimmable, fishable, and drinkable.'

In other words, most water pollution isn't from just a single point source of contamination, like a contaminated well or leaky sewerage pipe. Water pollution is caused overwhelmingly by general runoff of water from inhabited areas, bringing with it human contamination, such as sewage and chemical products. In agricultural areas, its brings contamination from livestock and agricultural material, such as fertiliser and decomposing crops. In addition, pollutants emitted into the atmosphere return to earth by gravity and also dissolved in rain, adding to the contaminants that runoff into the river system. These materials are often highly nutritious for bacteria and other causes of human disease. Runoff from such areas provides nitrates and other nutritive substances that enable massive bacterial and algal growths in rivers and lakes, to the detriment of the fish and natural wildlife there, as well as making the water unsafe to drink or even swim in. To clean up our rivers and lakes, we need to clean up the runoff water before it gets to the rivers and lakes. That's where Professor Endreny wants to use Mother Nature to fix Mother Nature. Besides studying traditional ways to combat

‘Our team builds models to find the origin and map the flow path of these pollutants, and identifies areas where trees could be planted to filter and clean the polluted water before it reaches our rivers, lakes, and oceans’



pollution, such as by restructuring the current and often out-dated and overloaded pollution treatment systems, he wants to plant trees so clean up our water.

## Landscaping – Connecting Humans to Nature

To environmental engineers, landscaping is connecting the built and natural environment towards a specific purpose, usually the improvement of the environment or a solution to some human problem related to nature. For example, if a town or city in an arid environment is plagued by flash flooding during the rainy season, the area may be landscaped by environmental scientists to provide canals and runoff reservoirs to channel floodwaters away from houses and businesses and green areas to absorb sudden rainfall. In agricultural areas with cyclic rainy and dry seasons, canals can be designed to provide water from adjacent areas to ensure consistent crop and livestock watering. In any case, modifying the landscape of an area is more than just making it pleasant on the senses – it can result in striking and long-range effects on environmental quality and human well-being.

Early in his training, Professor Endreny saw that landscapes could be better managed to allow for agriculture and urban development, as well as functioning waterways. 'I saw a chance to advance research and management on this problem by using new remote sensing products and computer hydrology model routines to identify and track the likelihood for pollution to enter receiving waters, and thereby guide planners toward better landscape management,' he explains. In 2002, he published a paper discussing the use of forest buffer strips for trapping nonpoint-source pollutants and protecting surface water quality. At the time, there existed few models designed to identify key areas for buffer installation and management, so he presented a conceptual model of polluted runoff dynamics for estimating watershed-wide forest buffer needs. The model took into consideration elevation and land-cover maps, along with calculations that represented basic nutrient runoff principles. In other words, you can calculate the nitrogen and phosphorus waste runoff from a specific area and determine where to plant trees to denitrify the polluting runoff before it reaches waterways.

What Professor Endreny and his team currently do is build models of problem spots around the world to find the origin, and map the flow path of various pollutants in order to identify areas where trees could be planted to filter and clean the polluted water before it reaches nearby rivers, lakes and oceans. 'The model uses proven yet simple scientific principles to describe the pollution flow path and the filtering process in order to balance accuracy and ease of use. It is our goal to create a model as the go-to tool for communities interested in participating in their land use planning,' he explains. And one of the end products of these models is the planting of trees in strategic positions so that, for example, nitrogenous wastes can be absorbed from runoff before it reaches local bodies of water.

## Megacities Mean Mega Benefits for Mankind – But We Cannot Forget the Environment

There are a number of theories about the origin of the British slang term for toilet – loo – but the most amusing for tourists is that it derived from the Anglicised shout of 'gardyloo' – from the French *regardez l'eau*



or ‘watch out for the water’ – that was shouted by servants as they emptied chamber pots out of upstairs windows into the street. Whether this is the true etymology or not, it underscores a problem that arises when humans concentrate themselves in small areas, like cities. Fresh water and food has to come from somewhere, since there are usually no fresh creeks or farms in the midst of large cities, and waste has to go somewhere. In past centuries, throwing waste into streets was the norm, and history is replete with stories of plagues and epidemics and the subsequent rise of modern civil engineering, sewerage treatment and trash collection. But the lesson is obvious – the larger the city, the more problematic the disposal of waste and other pollutants. You can’t just throw everything in the streets if there are millions of people living in the city. This is where the concept of a megacity is important and right up Professor Endreny’s alley.

Megacities are defined as metropolitan areas with a population in excess of ten million people. These can be single large city, like Shanghai, or metropolitan areas – so-called metroplexes or metropolises – where several smaller adjoining areas merge into one large confluence, like Tokyo. For Professor Endreny’s purposes, megacities are demographic epicentres in the production of livelihoods and market places. But because their populations are so large and so dense, their function – human wellbeing and livelihood – is jeopardised by pollution, climate change, and budget gaps limiting investments in education and healthcare. His research looks to quantify how existing and potential tree cover can lead to cleaner production, climate change mitigation and economic savings in these large population centres. He is interested not in, say, just New York or London, but in megacities across different continents and biomes. Different climates may mean different trees, but the science remains the same – use trees to absorb runoff pollutants before they reach the waterways and cause human problems.

Looking at Trees and Megacities All Over the World

The team has looked at multiple megacities and calculated the effect that planting trees would have on the millions of people involved. In a presentation at the July 2016 conference of the World Summit of Environmental Accounting, Professor Endreny presented research that scientifically calculated planting of trees could potentially contribute billions of dollars to the sustainable production of livelihoods in the megacity ecosystems. He and his team used a software tool called i-Tree, available for free at [www.itreetools.org](http://www.itreetools.org). This is a suite of computer programs developed to help communities inventory their tree cover and estimate the associated ecosystem services related to that tree cover. The tools use input data of tree structure, air pollution, weather, buildings, and economic pricing to calculate estimates of the tree-based ecosystem services of air pollutant reduction, storm water runoff reduction, building energy savings and carbon emissions avoided, and sequestration of carbon dioxide.

Professor Endreny’s team used the i-Tree tools to survey ten cities: Beijing, China; Buenos Aires, Argentina; Cairo, Egypt; Istanbul, Turkey; London; Great Britain; Los Angeles, United States; Mexico City, Mexico; Moscow, Russia; Mumbai, India; and Tokyo, Japan. They chose these cities because they span five different biomes, based on their annual average rainfall, high and low average temperatures, and native vegetation density and characteristics. They input data collected from each city and calculated the area of tree coverage and the effect that coverage had on local environmental and economic factors.



They found that the relative tree cover area of megacities was lowest in desert and Mediterranean biomes, such as Cairo and Istanbul, as expected. It was largest in Tokyo, also the largest city in area. Tree-based ecosystem benefits came to a median value of \$1.1 billion USD per year in total benefits, equivalent to \$2.7 million square kilometres per year of tree coverage, or \$76 USD per capita yearly for a megacity, and 0.3% of the megacity gross domestic product. Importantly, the team calculated that megacities can potentially increase these benefits by 85% by strategically increasing their tree cover. This could serve to better filter the city-generated pollutants and create a cleaner environment for human living and pursuit of human livelihoods.

Where is the Research Heading?

Looking to the future, Professor Endreny would like to improve upon his models to better predict locations with high denitrification potential in order to better predict the effects of intervention on filtering and removing nitrogen pollutants. He and his group intend to specifically map out urban flow paths within the micro-topography of city road systems so they can try to guide runoff into the green infrastructure, where the trees can do their work. If they can predict the build-up of nutrients and other pollutants in the landscape, they might be able to use alternatives of export coefficients and event mean concentrations as estimates of the pollutant load in any given area. Finally, they want to be able to incorporate future climate and land use predictions – how are the cities and farms going to expand – to simulate their effect on nutrients. Then they will be able to calculate where to plant trees now to manage the runoff in future. ‘Plant a tree, save the planet’ isn’t just about deforestation anymore. It’s about having a glass of clean water.



Meet the researcher

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Professor Theodore Endreny did his undergraduate studies at Cornell University in Ithaca, New York, in Natural Resources Management and received his B.S. in 1990, after which he served two years as a volunteer in the Peace Corps and Honduras Forest Service, and then worked 2 years as a research associate with the Environmental Law Institute in Washington, DC. He then earned an M.S. in Soil & Water Engineering in 1996 from North Carolina State University and a Ph.D. in 1999 in Water Resources Engineering from Princeton University in New Jersey. During his graduate training, he was funded by the US EPA Environmental Monitoring & Assessments Program and the NASA Graduate Students Research Project program. Professor Endreny joined the faculty of the SUNY College of Environmental Science & Forestry in 1999, where he is currently Professor and Chair for the Environmental Resources Engineering Department. He has authored or co-authored over 75 peer reviewed articles, reviews, and chapters, and more than 150 professional presentations. Endreny is a professional engineer licensed by the State of New York and a professional hydrologist licensed by the American Institute of Hydrology.

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FUNDING

U.S. Department of Agriculture Forest Service  
Mianus River Gorge Research Assistantship Programme

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## SHRINKING FISH STOCKS: THE EFFECT OF ENVIRONMENTAL SEX HORMONES ON IMMUNITY

The size of fish catches from rivers has reduced to alarming levels in many countries. **Professor Helmut Segner** of the University of Bern in Switzerland is studying the reasons why fish stocks might be dwindling. His research focusses primarily on the effect of sex hormones, and in particular oestrogens, on the immune systems of fish. These biomolecules are on the rise in water systems, for instance, due to the use of oral contraceptives and may be partially responsible for reduced fish populations.

### Where have all the fish gone?

The UK, Denmark, France, Norway and Switzerland are among the countries that have reported a recent decline in fish populations. In the 1970s, anglers in Switzerland reported over a thousand fish catches a year, and this reduced to annual numbers of under 600 in the late 1990s. There have also been many reports of fish malformations and reduced fish health. This reduction in fish numbers, including salmonoids (such as trout and salmon) and eels, which are regularly eaten by humans, is of huge concern. For this reason, researchers are on a mission to investigate what factors could be causing this significant drop.

### Oestrogens have widespread effects on physiology

Professor Helmut Segner and his team at the University of Bern explore the effect that sex hormones, such as oestrogens, can have on fish immune systems and their ability to modulate immune response. 'Originally, we were looking at the effects of endocrine disruptors on fish reproduction. These are

environmental compounds with hormone-like activities. Our focus was on compounds with oestrogenic activity, like ethinylestradiol which is the active ingredient in many contraceptives. We wanted to know how these compounds alter sexual development and impair reproduction of fish,' he explains when asked what led him towards this line of investigation.

These initial findings led the team to become interested in how these compounds might influence other aspects of fish physiology, aside from reproductive development. 'We observed that these oestrogenic compounds had a strong effect, not only on sexual development but also on fish growth, and this effect was mediated via interference with the growth hormone/insulin-like growth factor system,' Professor Segner tells us. At this time the team also discovered that the immune parameters of fish could be changed by exposing them to oestrogenic compounds. 'As my research team was also researching the immune response of fish to infectious pathogens, we became interested in the question of whether exposure of fish to oestrogenic compounds may compromise

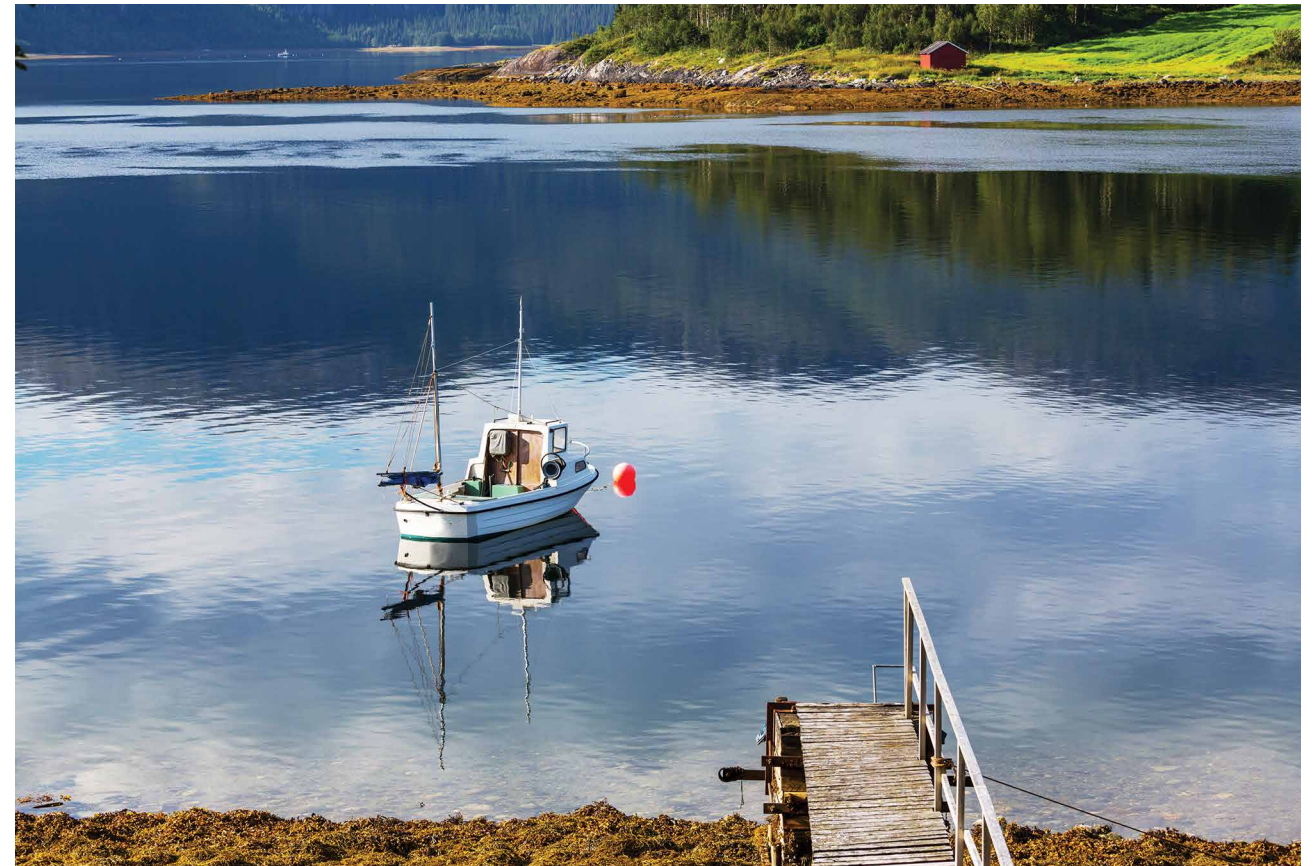
their immunocapacity and thereby enhance susceptibility to pathogens,' Professor Segner recalls.

### Females exhibit a stronger immune response

Research has shown that resistance to infectious pathogens exhibits gender differences in vertebrates. In the same way that the outward appearance opposite sexes may differ, so too does the way in which they combat infection. As Professor Segner explains: 'Generally, resistance, as well as tolerance towards viral, bacterial and parasitic pathogens, tends to be higher in female vertebrates than in male vertebrates. This is called the "female host supremacy paradigm".'

This sexual dimorphism, which has been well documented in mammals and birds, is believed to be caused by females being more immune-competent than males. 'There exists good knowledge on this in mammals, but virtually nothing on lower vertebrates including fish,' says Professor Segner. In humans for example, women show

**'We became more and more interested into the "non-canonical" effect of environmental oestrogens, that is, their interference with physiological target systems other than the reproductive system'**



stronger pro-inflammatory responses against pathogens than their male counterparts, but at the same women also have a better ability than men to dampen an overexaggerated inflammatory response to the pathogen. As a result, women have a much better chance of resisting infection. Professor Segner tells us about two of the factors that may contribute to this: 'Women generally have a larger relative spleen weight than males. They also tend to have higher white blood cell counts, higher immunoglobulin levels and higher CD4+ T-cell numbers.'

Despite the obvious advantages that this stronger immune system brings, there are downsides. For example, women reject transplanted tissues much faster than men and have a greater predisposition towards autoimmune disease. 'The female/male ratio of systemic lupus erythematosus SLE is 10:1, and of Grave's disease is 7:1,' Professor Segner adds.

### The immune system responds to sex hormones

So, what are the physiological drivers behind these differences in immunity between males and females? The fundamental factors responsible for the differentiation of the sexes are the sex hormones. Previous molecular and physiological studies have uncovered the effects that these hormones have on both the differentiation and function of immune cells. Androgens, such as testosterone (which exists in high levels in males), principally act as immunosuppressants, while oestrogens have been shown to enhance the immune system. Since females produce much higher levels of oestrogens, this is thought to be key to their higher immune-competency. 'This suggestion is corroborated by the fact that postmenopausal women, in parallel to the decline of oestrogen levels, also lose their immunological advantage against infections, but at the same time suffer less from

autoimmune diseases,' Professor Segner explains. 'In addition, immunity and disease expression of women correlates with their reproductive status (i.e., oestrogen levels), for instance, SLE can undergo remission during pregnancy.'

### Evolution of the immune system

Professor Segner and his colleagues want to find out if these immune actions of sex hormones are relevant in fish as well as mammals. They are also interested in understanding what lies behind this immune cell response to sex hormones. He asks the question: 'What is the evolutionary sense of this?' One hypothesis is that the sex hormones have immunomodulating activity because the organism has to deal with resource limitations for both the immune and reproductive systems – two highly valuable fitness components of animals. 'Life history theory proposes that organisms are not



selected for optimisation of either reproduction or immunity, but that evolution selects for the optimal allocation of the limited resources among fitness-associated traits,' Professor Segner explains. 'Nutrient and energy resources are limited and cannot sustain all life history traits at maximum levels. This implies that organisms have to make compromises in the allocation of resources among competing traits, and this implicates that they correlate negatively or "trade-off"'

Life history theory also explains why the effects of sex hormones may differ between males and females. 'As proposed in Bateman's principle, females gain fitness through increased survival and longevity, while males gain fitness by mating with many females,' Professor Segner explains. 'As a consequence, males invest less in immune defence and thus have lower life expectancy.' Additionally, males of most vertebrate species are less invested in parenting their young to increase the offspring's chance of survival. Instead, they devote their time to courting females and competing with other males to increase their mating success. In contrast, females cannot increase their reproductive success by mating with more than one male. 'They are choosy in mating and invest heavily in their offspring and, thus, future reproduction. In line with this, females show higher investment in the immune system in order to support survival and longevity and to increase the lifetime reproductive success,' says Professor Segner.

Understanding the effect of oestrogens on fish immunity

When embarking on their initial investigations, Professor Segner and his team found that very little was known about the effect of oestrogens on the immune systems of fish. First, the team needed to determine if fish immune cells are indeed influenced by oestrogens. To do this, they analysed the cells of rainbow trout to see if oestrogen receptors are present using Reverse Transcription Polymerase Chain Reaction (RT-PCR). Since this technique is used to detect RNA expression, the team used it to find RNA sequences that code for the creation of oestrogen receptors. Remarkably, all four nuclear oestrogen receptors (ERα1, ERα2, ERβ1 and ERβ2) were discovered in the studied immune cell populations. The presence of nuclear oestrogen receptors in other fish species has now also been identified, as have membrane-bound oestrogen receptors.

The team then investigated how exposure to oestrogens can modulate immune gene expression and immune functions in fish. This was done both in isolation and in the presence of pathogens. Professor Segner tells us about the importance of performing these experiments in the presence of a pathogen: 'It is often the case that an immunomodulatory effect becomes evident only when the immune system is activated, and not in the resting immune system.' They exposed rainbow trout either to oestrogens alone, or to a combination of oestrogens and a pathogenic parasite. Using a microarray approach, they discovered that when exposed to both stressors, parasite and high oestrogen levels, the fish response differed to then exposed to a single stressor alone. For example, some genes were downregulated (less expressed) when exposed to oestrogens, but in the presence of the pathogen they were upregulated (more expressed), while others remained downregulated.

The challenge experiment

The team needed to prove that oestrogenic substances had an impact, not only on immune parameters but also on the immune capacity of fish. In particular, they needed evidence to show that oestrogenic substances, endogenous or exogenous toxicants, can

‘Fish are complicated because they possess up to four nuclear oestrogen receptors (ERα1, ERα2, ERβ1 and ERβ2), and we found them all to be present in the various immune cell populations of rainbow trout’



have a compromising effect on fish immune systems, making them more susceptible to succumbing to disease. They devised a challenge experiment, using rainbow trout that they exposed to a pathogen called Yersinia ruckeri, which causes the enteric red mouth diseases of salmonids. The development of the disease and survival rates were then compared between a control group and an oestrogen-treated group. Unlike us humans, the fish exposed to oestrogen were significantly more susceptible to the parasite than the control fish.

Future work

Professor Segner's work is ongoing as he seeks to further understand evolutionary factors that give rise to an immune-reproductive trade-off. This interaction has relevance for both animal and human health, and sheds light on the immunological risks associated with exposure to endocrine disruptors. 'In the case of high fish mortality in rivers, the observer may blame pathogens as being responsible. But, the real cause would be the combined effect of the oestrogenic and the pathogenic stressor. Given the fact that, despite improved water quality that we now observe in many river systems, we still have a decline in fish populations, it is likely that these effects are not monocausal but arise from the cumulative impact of multiple stressors,' he concludes.



# Meet the researcher

Professor Helmut Segner  
Head – Centre for Fish and Wildlife Health  
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Professor Helmut Segner is Head of the Centre for Fish and Wildlife at the University of Bern, Switzerland. He completed his Master's degree in Chemistry and Biology and a PhD in Biology at the University of Heidelberg in Germany. He then went on to carry out postdoctoral research at the same university before taking a post as a research scientist at the Tetra Fish Food Company. Before arriving at his current position, Professor Segner was Assistant Professor at the University of Karlsruhe and then Head of the Aquatic Ecotoxicology Research Group at the Helmholtz Centre for Environmental Research in Leipzig. He also acts as an Associate Editor for the journals Aquatic Biology and Fish Physiology and Biochemistry, has published over 200 peer reviewed articles, and serves on a number of national and international advisory committees.

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FUNDING

Swiss National Science Foundation SNSF  
European Commission

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## GROWING BETTER SALMON: BALANCING ECONOMICS WITH ENVIRONMENTAL IMPACT

Aquaculture – growing fish or other aquatic species in captivity – is an important strategy for meeting the increasing demand for seafood from a growing human population, while also preserving wild fish stocks. However, aquaculture can also have negative environmental impacts. Researchers at the Universities of Windsor, Waterloo and Western Ontario in collaboration with industry and government partners are investigating ways to advance salmon aquaculture using methods that increase productivity, while reducing environmental impact. The implications for sustainable and profitable Chinook salmon farming in British Columbia and around the world are exciting.

According to the Canadian Department of Fisheries and Oceans, aquaculture represents about a third of Canada's total fisheries value and is growing rapidly; the value of aquaculture production has increased by 63% over the past ten years, to \$962 million currently. As much as \$634 million of this is attributable to salmon farming. Most salmon aquaculture focuses on Atlantic salmon, but, as Dr Daniel Heath, University of Windsor, explains: 'the culture of Pacific salmon for west coast aquaculture has great economic potential due to niche market price advantages and higher disease and parasite resistance.' He and his research team are investigating ways to improve Chinook salmon farming.

### Chinook Salmon

Chinook salmon are native to the Pacific northwest and are big fish, maturing close to 1 metre-long and ranging from 18 to 55 kg in weight. As with other species of salmon, they are anadromous, meaning that they migrate from salt water to the freshwater streams and rivers where they were born in order to mate and lay their eggs. Chinook salmon are listed on the Endangered Species List in the United States, and several Chinook fisheries along the Pacific coast in the U.S. and Canada are limited by weak numbers. The fishery suffers from a range of threats, from habitat degradation to climate change. These low numbers of Chinook salmon are particularly



concerning because the fish serve an important role in the marine and freshwater ecosystems by providing food for a variety of wildlife (such as bald eagles and grizzly bears), as well as contributing important nutrients and organic matter back to the freshwater and saltwater environments. They also hold cultural importance to their namesake, the indigenous Chinookan people of the Pacific Northwest.

### The Research Team . . . and a Whole Lot of Fish!

The research team is an experienced group of seven scientists at the University of Windsor, University of Waterloo and Western University in Canada. Dr Heath has a Ph.D. in Genetics from the University of British Columbia and an M.Sc. in Biology from McGill University. He has served as Principal or Co-Principal research scientist on numerous grants to advance research around aquaculture, fish biology, animal science, and ecology. His expert research team includes Dr Dennis Higgs (University of Windsor) who focuses on sensory ecology of vertebrates, Dr Oliver



CREDIT: Ann Heath

Love (University of Windsor) who studies physiological traits in a variety of species across different ecosystems, Dr Brian Dixon (University of Waterloo) who is an expert in fish immunology and disease, Dr Christina Semeniuk (University of Windsor) who studies adaptive behavioural variation in social groups of animals with a focus on resource utilisation, Dr Bryan Neff (Western University) who focuses on the behaviour, genetics and ecology of fishes and Dr Trevor Pitcher (University of Windsor) who combines evolutionary ecology, reproductive biology and conservation biology, to study fishes from the Great Lakes and the Pacific coast of Canada.

Dr Heath and his team aim to reduce the environmental impact and increase the profit associated with farming Chinook salmon in the Pacific northwest by developing high performance Chinook salmon stocks that gain weight easily on less food and have improved immune function. The goal is to produce fish that will require less feed and fewer chemicals and antibiotics for disease control, thus significantly reducing both costs and pollution. Their current research, funded by the Natural Sciences and Engineering Research Council of Canada, involves multiple integrated research topics designed to culminate in high performing strains of Chinook salmon that can form the backbone of a profitable and environmentally sustainable Chinook aquaculture industry in the Pacific northwest.

As one can imagine, the research involves a lot of fish! The research team conduct their trials at Yellow Island Aquaculture, Ltd., an organic salmon farm on Vancouver Island and one of the industrial partners in the project. Juvenile fish were housed in 240 family tanks – 200 litres in size, and then transferred to several 5x5 metre saltwater net pens as they grew. Multiple groups of fish (and hence tanks and pens) are necessary to test the impact of different variables on the fish. A subset of 16,800 of the fish being studied also have a small electronic identification tag called a PIT tag that allows the researchers to track their growth and development in extreme detail.

### Hybrid Vigour

Hybrid vigour is foundational to the research team's efforts to create high performing Chinook salmon breeding stock. Hybrid vigour describes the superior qualities that arise from cross breeding genetically different plants or animals (you might be familiar with this if you are a gardener growing prolific hybrid vegetable strains purchased from your local garden supply centre). Dr Heath and his team are working to achieve hybrid vigour in Chinook salmon stocks by crossing domestic and wild salmon. These hybrids have good potential for achieving higher growth and survival rates and better feed efficiency than either of the parent strains. By carefully studying these hybrids under different environmental conditions, researchers are

able to continually select and breed for a highly productive fish.

This is no small task – creating these hybrid fish stocks involves taking 60,000 eggs from mature Chinook salmon females and collecting sperm (also called milt) from wild populations across Vancouver Island, a task only possible with the guidance of Dr Bob Devlin, a Department of Fisheries and Oceans Canada researcher. A portion of the sperm collected was cryopreserved for use in creating the experimental hybrids.

As the research team carefully builds the breeding stock, they monitor multiple variables closely – from growth and metabolic rates to aggressive behaviour patterns, energetic physiology, immune response, survival and flesh quality.

### A Salmon Treadmill of Sorts: Assessing Growth and Neurobiology

Growth is measured not just in length and weight of the fish, but also according to the presence of growth and stress hormones and in terms of feed conversion and metabolic rates. Feed conversion efficiency (how much food it takes to grow to a specific size) is measured as a ratio of feed consumed to body size. How is metabolic rate measured? To obtain this measurement, a sample of 10 fish from each stock are put in a swim tunnel where a respirometer records their oxygen consumption at rest and at maximum





sustained swimming speeds. Because water temperature impacts fish performance, swim tunnel measurements are taken across a range of water temperatures that correspond to climate change prediction models. To get a clear picture of growth rates throughout the full life cycle of this anadromous fish, the research team monitors growth in both freshwater and saltwater environments. Because captive environments have been shown to have the potential to alter the brain in a way that reduces efficient physical growth, the team also performs complex analyses on samples of brain tissue and relates those measurements to adaptive behaviour patterns in the fish.

#### Salmon Aggression: Assessing Behaviour and Endocrinology

Why do we care how fish behave when in groups? Dr Heath explains that fish behaviour 'is a key determinant of fish performance in aquaculture.' Aggression between fish can cause injuries and increases in cortisol (a stress hormone that, when elevated, can cause a multitude of physical problems). Aggressive behaviour can also reduce the growth rates among individual fish that have trouble accessing food, and the frequent speedy movements of fish either acting as aggressors or getting out of the way of an aggressor increases the fish's metabolic rate, in turn decreasing its food conversion efficiency. Interestingly, behaviour patterns related to aggressive behaviour have been shown to be a product of both genetic traits and the captive environment experienced by the individual. Therefore, it is possible to reduce aggressive behaviour through breeding.

In order to analyse and quantify fish behaviour, the research team uses both video analysis and blood samples (the latter detects levels of hormones, such as cortisol and testosterone, that are known indicators of stress response and social dominance in fish). They also analyse gene expression in brain tissue to identify the mechanisms of genetically-based aggressive tendencies.

#### Survival Rates, Pathogens and Stress

All fish can become susceptible to disease or parasites, but in crowded pens of farmed fish, the risk of economically devastating disease outbreaks is increased. Globally, aquaculture losses due to disease cost billions of dollars. Antibiotics and pesticides are costly and harmful to the environment, so developing a fish with a robust immune system is a top priority. Dr Heath's team is measuring immune response in their fish stocks by monitoring cytokines (small proteins that act as signals to

cells and modulate immune response) as well as antibody production and granulocytes (a specific kind of white blood cell). Because stress hormones play a role in fish health, the relationship between stress hormone levels and survival is being documented. Analysis of DNA from both the salmon and their pathogens allows the researchers to identify pathogens and observe the relationships between genetic diversity and survival rates.

#### Immune Function

Further in-depth analysis of immune function is also ongoing and involves challenging fish by exposing them to disease via vaccine and bath exposure (in which fish are transferred to tanks containing disease). In addition to monitoring for survival (genetic variation among the fish means that some fish have better survival rates than others), the researchers analyse blood samples, along with spleen, gill and muscle tissues to monitor immune response. Finally, genotyping allows the scientists to examine individual DNA variation that may contribute to improved immune response and ultimately, survival.

#### Flesh Quality

High quality flesh is (of course!) an imperative for a successful salmon farm. However, flesh quality can be negatively impacted by stress associated with high density pens. It also tends to deteriorate as the fish ages. The research team monitors flesh quality (including pigment, fatty acid content and muscle composition) in relation to diet, the presence of stress hormones and maturation rates. These data help them better understand the optimum living environment for the stock as well as the optimum age for harvest.

#### Bringing it All to Market: Economic Assessment

The final piece to the puzzle involves calculating the economic potential of the newly developed fish stocks. To that end, the research team will combine all of its performance parameters, quantify the cost-benefit relationships associated with each hybrid strain developed through the research project, and produce a profitability analysis comparing Chinook salmon with the more commonly reared Atlantic salmon. The researchers expect that the analysis will help Canada's salmon farming industry diversify, increase profits and reduce negative environmental impacts.



## Meet the researchers

#### Dr Daniel Heath

Dr Heath's (U Windsor) role is to correlate survival and growth with gene expression profiles. He has extensive experience with collaborative applied and academic aquaculture research, including molecular and quantitative genetic analyses of salmon. He is currently conducting research investigating the genetic contribution to migratory and maturation variation in salmon and trout and has worked in, and collaborated with, the Canadian salmon farming industry for over 30 years.

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#### Dr Oliver Love

Dr Love's (U Windsor) role is to evaluate hormonal mechanisms affecting performance (growth and survival). He has extensive experience measuring and interpreting physiological biomarkers of growth, performance and environmental stress. He is currently conducting NSERC- and Canada Research Chair-funded research investigating the mechanistic links between human-induced environmental stressors and performance in vertebrate systems, and has published on the mechanistic role of developmental and reproductive stressors in driving individual variation in fitness.

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#### Dr Bryan Neff

Dr Neff's (U Western Ontario) role is to evaluate feed conversion efficiency and the contributions of metabolic rate and activity to this trait. He has extensive experience studying the behavioural, ecological and evolutionary factors affecting life history traits in fishes. He is currently conducting NSERC-funded research to understand the genetic architecture of fitness in fishes, and to help restore Atlantic salmon to the Great Lakes.

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#### Dr Dennis Higgs

Dr Higgs' (U Windsor) role is to evaluate the neurobiology of growth. He has extensive experience in the growth and development of fish and neural correlates to performance. He is currently conducting NSERC-funded research on the growth performance of Chinook in aquaculture settings as well as NSERC-funded research on the effects of human-induced stressors on sensory performance and applied work utilising behavioural and neural responses of invasive species to improve ongoing remediation efforts.

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#### Dr Christina Semeniuk

Dr Semeniuk's (U Windsor) role is to correlate survival and growth with behavioural profiles at the individual and population levels. She has a background in behavioural ecology and has NSERC-funded research to examine the mechanistic drivers and functional outcomes of behavioural variation of animals experiencing human-induced rapid ecological change. Her program provides managers with necessary information to aid in the screening of optimal phenotypes for aquaculture, captive breeding, and conservation-related initiatives.

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#### Dr Trevor Pitcher

Dr Pitcher's (U Windsor) role is to evaluate flesh quality in relation to growth rates and survival to harvest size by quantifying carotenoid, lipid, fatty acid and muscle composition amongst the strains. Pitcher has extensive experience with Chinook salmon, including NSERC funding to study how incorporating ecological and evolutionary principles into breeding designs can improve aquaculture and reproduction in the context of evolutionary ecology.

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#### Dr Brian Dixon

Dr Dixon's (U Waterloo) role is to evaluate disease resistance and immune function to optimise Chinook salmon survival. He holds a Tier I Canada Research Chair in Fish and Environmental Immunology and was awarded the 2016 Wardle medal by the Canadian Society of Zoologists for lifetime achievement in fish immunology. His research program is focused on the immune response of fish to pathological and environmental stressors and his lab also has over 20 years in teleost fish MHC research, fish molecular immunology techniques and the development of antisera for examining expression of immunological molecules.

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## EXPLORING OUR OCEANS

Modern oceanography is said to have begun with the Challenger Expedition that took place between the years of 1872 and 1876. The naval ship used for the adventure – the HMS Challenger – was kitted out with several laboratories on board, containing microscopes, chemical apparatus, trawls and dredges, thermometers and water sampling bottles, specimen jars and sounding lines. Using this equipment, the scientists on board could collect data on a wide range of ocean features, such as seawater chemistry, ocean temperatures, currents, marine life and seafloor geology. Under the scientific supervision of Charles Wyville Thomson of the University of Edinburgh, and with a crew of more than 200 people on board, the ship sailed for over 130,000 km, surveying and exploring the global ocean. Over the course of the four-year expedition, more than 4,500 new species of marine life were discovered and one of the deepest parts of the ocean – the Marianas Trench – was found.

One hundred and forty years later, and the science of oceanography has evolved tremendously. Whereas scientists on the

HMS Challenger used a marked rope (a sounding line) with a weight attached to measure ocean depth, nowadays we can use multibeam echo sounding, which uses sonar pulses to map large areas of the sea floor at once, with remarkable accuracy. The suspended stoppered bottles that were used to collect deep-ocean water samples to be later analysed for salt and mineral content, have now been replaced with real-time monitoring platforms built to withstand the crushing pressure of the deep ocean. Making observations at fixed points in the ocean over long periods of time is essential for increasing our knowledge of how our oceans function, and discovering how they are changing with our changing climate. In this section of the edition, we highlight the work of several modern-day oceanographers who are lucky enough to explore the oceans for a living.

Bringing oceanography into the internet age is Dr James Potemra, along with his colleagues at the University of Hawaii's School of Ocean and Earth Science and Technology. The team are behind the creation of the ALOHA Cabled Observatory



– a high-tech suite of instruments on the sea floor, just north of the Hawaiian Islands. These instruments measure pressure, temperature, salinity and ocean currents, in addition to recording sounds with the use of a hydrophone. The observatory sends data to shore through an undersea cable provided by the telecommunications conglomerate AT&T, to be published ultimately on the World Wide Web for scientists and the public alike to see and use. Also using a cabled observatory to probe the depths of the oceans are Dr Karen Bemis (Rutgers University), Dr Darrell Jackson (University of Washington), and Dr Guangyu Xu (Woods Hole Oceanographic Institution). In the second article of this section, we describe the Cabled Observatory Vent Imaging Sonar, or the COVIS, which is capable of high-resolution sonar imaging, offering the researchers a way to look into the abyss. In particular, the team use this acoustic imaging to obtain a 3D view of oceanic hydrothermal vents, their plumes and their surroundings.

Next we introduce Professor Robert Mason, a marine scientist at the

University of Connecticut, who studies how mercury levels in the oceans are changing due to climate change. He has collaborated internationally to study mercury levels in the ocean's surface and the lower atmosphere. Studying mercury exchange at the sea surface is of huge importance, as it helps us to understand the impact of human activity on mercury contamination in the seafood we eat. Continuing with the theme of how anthropogenic activity is affecting Earth's marine habitats, we highlight the work of Professor Gordon Taylor at Stony Brook University, who is working to understand the complex dynamics that govern water chemistry by studying microbes. By leading and participating in several research expeditions every year, Professor Taylor and his team investigate the decline of dissolved oxygen in the ocean due to climate change, and how this affects microbial life. This important research leads us nicely on to our next section, where we explore the evolution of life on earth.





## INVESTIGATING WHAT GOES ON AT THE BOTTOM OF THE DEEP BLUE SEA

Oceanographer **Dr James Potemra** and his colleagues at the University of Hawaii's School of Ocean and Earth Science and Technology (SOEST) monitor one of the most remote places on Earth – the bottom of the ocean – with the ALOHA Cabled Observatory, a high tech suite of instruments on the sea floor north of the Hawaiian Islands.

### Probing the Mysteries of the Deep

What do you think of when you think of the ocean floor? Maybe you find yourself looking out of the viewport of the submarine Nautilus at Captain Nemo and his hard-hat divers, as they trudge slowly along the ocean landscape. Or perhaps you find yourself in the deep-sea mining vehicle Big John, roving the Pacific seafloor while Dirk Pitt sits at the controls singing Minnie the Mermaid. Wherever your mind wanders, it probably doesn't wander much beyond the realm of fiction. What do we really know about the sea? Look at the news these days and you are much more likely to see reports of, say, the 2020 Mars Rover, the International Space Station or the latest meteor shower. You usually don't hear much about the ocean or oceanography, except perhaps for an article here or there about a beached

pod of dolphins or an advertisement for the next season of Shark Week. Although over 70% of the Earth is covered with water, it may appear that not much attention is paid to our oceans. But Dr Potemra and his colleagues do – and they have been for over a decade. Today, they use the ALOHA Cable Observatory, or ACO, to watch the seafloor over the Internet!

The ACO, which is funded by the National Science Foundation, comprises a suite of instruments, cumulatively about the size of a Volkswagen Beetle, on the sea floor north of Oahu, Hawaii. These instruments include a range of ocean-monitoring devices that measure pressure, temperature, salinity and currents, but also include a hydrophone, to measure sounds in the ocean, and cameras and lights. All of these instruments send data to shore via an undersea cable provided



by AT&T, to be published ultimately on the World Wide Web for scientists and the public alike to see and use. The system is unique not only because it is the deepest such observatory in the world, but also because it provides a platform – including electrical power and Internet – at one of the most remote places on earth. Data from the ACO are wide-ranging, and scientists like Dr Potemra use these data to study things like marine mammal presence (from acoustics), seismic activity, deep ocean thermal variability and circulation, and much more. As one of the most under-sampled regions in the world, any new information coming from the ACO represents a significant advancement in our understanding of the ocean.

### Almost 20 Years in the Making

Since it seems humans can more easily walk on the Moon than on the floor of the deepest ocean, collecting data on the world's oceans has been tedious and frustrating. Historically, shipboard spatial surveys and maps of marine characteristics conducted by early oceanographers revealed some secrets of the seas. Think of Charles Darwin and the sailing ship HMS Beagle, cataloguing islands and wildlife in the Pacific Ocean. However, time series measurements were required to explore the variations of the ocean at a single location. Repeated shipboard observations at particular sites provided access to such variations, but the temporal sampling is limited by the duration capabilities of the ship and crew. In addition, the characteristics of the ocean at any particular spot may change between successive lowerings of instruments, since the ocean contains large amounts of energy varying at high frequencies. Such measurements were often only accurate for a short time.

To cope with the sampling problems – like having a ship and crew anchored in a particular spot for weeks at a time – engineers packaged sensors (e.g., temperature, pressure, acoustic, seismic) into pressure-resistant cases along with batteries and tape recorders. The sensors could be dropped and left and recovered later. While this strategy provided great insight into subsea conditions, the records were always limited by either battery capacity or data storage. Understanding the dynamic processes of the ocean and sea floor required long-time series to reliably quantify deep sea conditions. Oceanographers needed a paradigm shift in their methods of observing

**The ACO enables continuous real-time measurements of the deep sea sustained for long periods. The measurements are available nearly instantaneously, which is a vast improvement over traditional oceanographic instruments, with their limited battery capacity and measurements that are not available until the instrument is recovered from the sea floor.**



the ocean. Enter the Conference on the Scientific Uses of Submarine Cables, in Honolulu, in 1990. The discussion ensued: How can we use retired but still useful undersea cables from telecommunications companies to carry data from subsurface oceanographic sensors?

### Hawaii Ocean Timeseries

The ACO is located at Station ALOHA, an ocean location 100 km north of Oahu, Hawaii, nominally a circle 12 km across. It is the study site of the Hawaii Ocean Timeseries (HOT) program. HOT is focused on studying processes that control the distributions and cycling of elements in the sea – with specific focus on carbon – in sufficient detail to provide predictive understanding on how global scale changes to ocean climate influence biogeochemical transformations. To achieve this broad objective, the programs sought understanding of the linkages between seasonal, interannual and long-term (multi-decadal) variability and trends in ocean physics, chemistry and biology. Processes underlying physical and biogeochemical temporal variability were also studied, as well as the role of

physical forcing on carbon fluxes, including rates of biologically-mediated carbon transformations, air-sea CO<sub>2</sub> exchange and carbon export.

HOT was started in late 1988 and relies primarily on traditional oceanographic protocols, such as quasi-monthly ship cruises to make the measurements. However, it attracted a multitude of related science projects because of its location and comprehensive scope. It is the combination of all these activities that makes Station ALOHA such a unique and attractive science study site. Subsequently, a surface mooring was placed near Station ALOHA to provide much needed atmospheric observations (e.g., wind speed and rainfall), the Woods Hole Oceanographic Institution/Hawaii Ocean Time-series Site (WHOTS) mooring.

Then, in 2002, a portion of the retired AT&T HAW-4 submarine fibre optic cable was donated to the University of Hawaii – actually, it was sold to the University for \$1. HAW-4 had been one of several first generation fibre optic systems installed in 1988. It has 3 fibre pairs (one is spare), each of which is capable of transmitting at 295 Mb/s.



The cable originally ran from the Makaha Cable Station, Oahu, Hawaii to Point Arena Cable Station, California, a distance of 4260 km. In the 60 repeaters spaced every 70 km or so, the light signal was converted to an electrical signal, amplified, and changed back to light to continue on. In early 2007 the US Navy graciously cut the cable, moved the Hawaii end to Station ALOHA, about 100 km north of Oahu, and attached an initial set of sensors to the end of the cable. This provided continuous, real-time sound from the site to shore. The signal is then transmitted directly to waiting scientists like Dr Potemra and his colleagues at the UH campus. The piece that connects ACO to Oahu is 237 km long. The other segment that terminates in California is much longer, roughly 4000 km.

**The perfect combination – and a drone!**

In June 2011, a general purpose node was deployed on the site. This provided an interface into which instruments could be plugged and through which data and power could flow. Since then, two more cruises using deep diving remotely operated vehicles (ROV) – in other words, undersea robots – have gone to the ACO site to deploy additional sensors and instrumentation. The ACO site is on the sea floor approximately 5 km deep. The extreme pressures and its remote location make work here challenging, but the real-time monitoring coupled with the surface mooring and HOT program allow for unique, wide-ranging studies of the ocean and earth’s climate.

Physically, the ACO is made up of a set of different platforms, all resting on the seafloor and connected by cable. The undersea cable from the shore is terminated at the ACO ‘junction box’ (JBOX) that converts the telecommunication protocols to 100 Mb/s Ethernet. A hydrophone experiment model (HEM) and pressure sensor are mounted on the JBOX. Following the cable from the JBOX, the next platform is the main component of the ACO, the so-called OBS. Like the JBOX, the OBS has instruments mounted on its frame, including sensors for temperature/salinity, ocean current and pressure. In addition, the OBS provides the outlets for additional components. These outlets, or ports, allow for additional sensors to get power and data links. At present there are two Basic Sensor Packages (BSP-1 and BSP-2), two video cameras, and several lights connected to the OBS. Despite the best of engineering, due to the extreme pressures and otherwise harsh environment, several of the instruments have failed, most notably the light system, so the available video footage is limited.

However, there are many other data streams coming from the ACO in real-time. These include total water column pressure, near bottom temperature, near bottom salinity, north/south and east/west current speeds from about 100 meters off the bottom to the near sea floor, ambient ocean sounds, turbidity, fluorescence (which is indicative of chlorophyll content). Archived data include several months of underwater video and additional hydrophone data.

**It’s all about meeting challenges**

The ACO facility represents advances in many different areas. First, there is the challenge of working in the ocean environment, where salt and water can be extremely hard on electrical components. Special care needed to be taken in designing the instrument modules to prevent leaks and corrosion.

Second is the challenge presented working at great depths, where there is a complete absence of natural light and the pressures are extreme



(almost 7000 psi). At 4728 m, this is currently the deepest operating cabled observatory with ‘plug and play’ capability. The ACO team was able to overcome these pressures with pressure tolerant housings and specially designed cables and connectors that allow instruments to plug into the ACO directly on the ocean floor using unmanned submersibles.

The third challenge involves the cyber-infrastructure aspect of real-time data systems. Since the site is so remote and a single cable is involved, bandwidth and Internet port management is critical. The ACO has been able to manage these and still provide real-time data, including video, that is streamed directly to the web. In fact, anyone with Internet access can view ACO data from the comfort of their own couch by logging into <http://aco-ssds.soest.hawaii.edu/dataDisplay.php>.

It is pretty clear that Dr Potemra and his colleagues have met these challenges. The ALOHA Cabled Observatory has succeeded and will continue to shed light on the science of the seafloor and the water column above for many years to come.

**The Agenda – Now and for the Future**

There are several on-going and future scientific studies using the ACO. The original concept was to demonstrate the ability to put in place a deep-sea observatory that was directly connected to a base on land so as to provide real-time access to the sensors and power to the instrumentation. That has been accomplished. On-going observations includes studies of deep-sea biology, abyssal circulation and mixing, and acoustic signatures of earthquakes, ships, marine mammals, wind waves and rain. Other proposals in progress deal with predator-scavenger community dynamics, benthic community response to changing climate and food supply, and a mooring system sampling the full water column on a rapid, sustained and adaptive basis to address the carbon cycle, water column dynamics and mixing, and bio-physical interactions.

So now the ACO is now actively seeking scientists to take advantage of the ACO platform for their studies. Some proposals have focused on monitoring the benthic community with a bottom ‘rover’ that would be powered by the ACO and would study the sediments around the site. NASA has the Mars Rover to dig up Barsoom; why can’t ACO have a rover to take samples on the ocean floor? It’s time that stories of the seas and oceans stop taking a backseat to news about space.



# Meet the researcher

**Dr James T. Potemra**  
Manager, Asia-Pacific Data-Research Center  
International Pacific Research Center  
Hawaii Institute of Geophysics and Planetology  
University of Hawaii  
USA

Dr James T. Potemra received his BSc in Physics in 1986 from the Stevens Institute of Technology in Hoboken, New Jersey, and an MSc in Oceanography in 1990 from Florida State University. He was then awarded his PhD in Oceanography from the University of Hawaii (UH) Manoa in 1998. After completing a postdoctoral fellowship at the University of Washington, Dr Potemra returned to UH Manoa to the International Pacific Research Center (IPRC), where he is currently the Manager of the Asia-Pacific Data-Research Center. In addition to this, he is a faculty member at the Hawaii Institute of Geophysics and Planetology, where he teaches students about accessing and analysing large sets to understand climate variability. Dr Potemra’s research interests include general ocean circulation and its relationship to climate, and the various ocean circulation processes in the western equatorial Pacific and eastern Indian Ocean and their connection. Dr Potemra has authored or co-authored more than two dozen papers in refereed scientific publications dealing with geophysics and oceanography.

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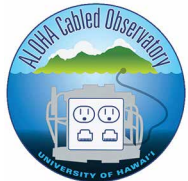
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**FUNDING**

NSF





# THE LAST UNEXPLORED PLACES ON EARTH

It takes a multidisciplinary approach involving physics, chemistry, biology and geology to uncover the mysteries of the least explored and understood places on Earth.

## A blind sighted look into the ocean depths

Earth is a water world. Although oceans cover 71% of its surface, they are notoriously difficult to explore and therefore humanity knows little about what happens under the water. In this era of information and scientific discovery, the rapid pace of research is swiftly closing the last gaps in the human knowledge of our home planet. Yet surprisingly, many things about the dynamics of the oceans remain unknown, or not understood well enough.

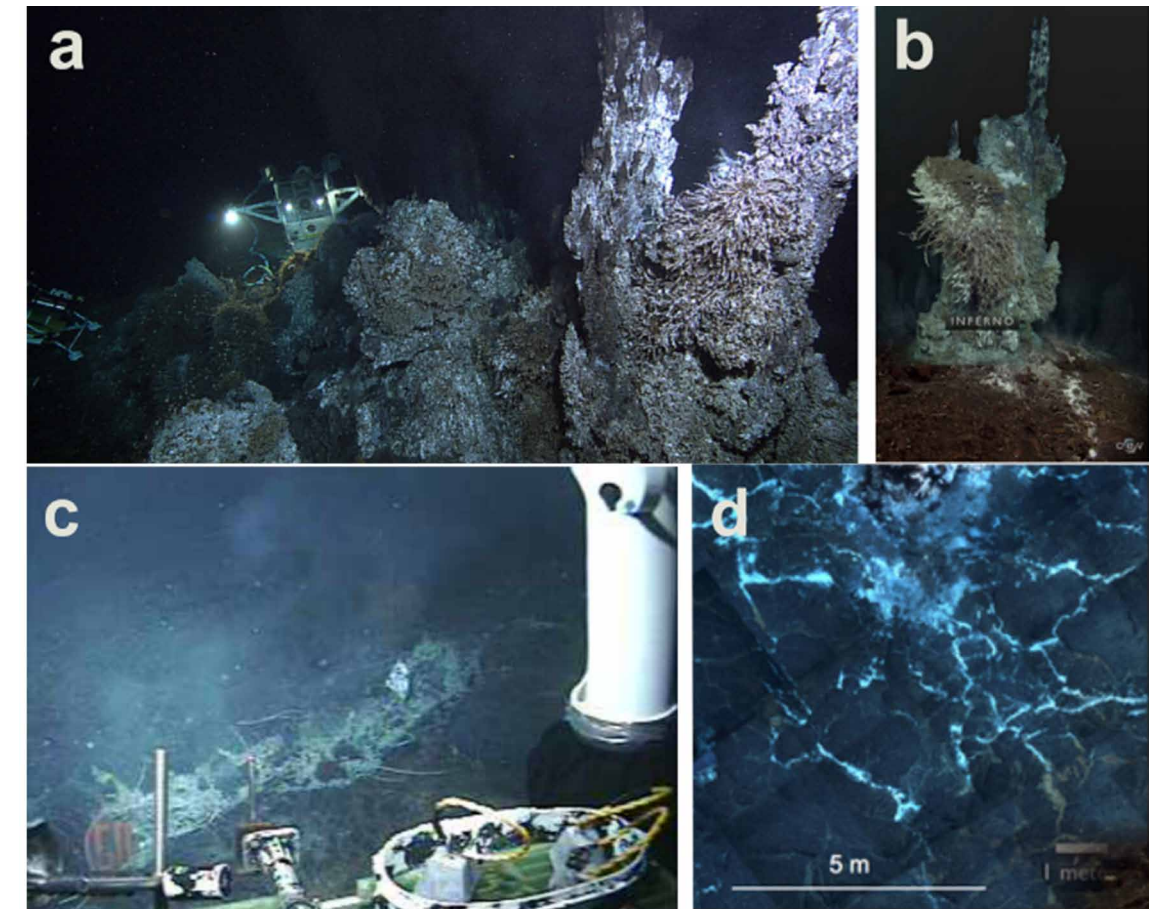
The oceans are home to 2.2 million species, both large and small. However, it takes special equipment and a lot of determination to explore their depths, as the abyssal regions and trenches pose exceptional difficulties and dangers for even the most daring of explorers. In some of these interesting places, the pressure climbs to a crushing eight tonnes per square inch. Moreover, sunlight only penetrates down to about one kilometre from the surface under the right conditions, but more often there is no significant light beyond the first two hundred metres. The bottoms of the oceans are cold, dark places – perilous for humans, but teeming with the life of benthic ecosystems and having an important impact on the planet's climate.

Achieving a good understanding of the planet's salty bodies of water and the crust at their bottom is not easy. In fact, knowledge from multiple scientific areas must be used to properly deal with the challenges of underwater research and discovery because of the many barriers posed by

COVIS on afterdeck of the University of Washington Research Vessel THOMPSON for the September 2010 deployment cruise. From left, Vern Miller, mechanical engineer, Russ Light, leader of engineering team, Peter Rona, organizer and leader of the collaboration.



**‘Deep-sea hydrothermal vents are important because they export large amounts of heat and chemicals to the ocean and support unique benthic ecosystems. Our understanding of the ocean will remain incomplete without fully understanding hydrothermal vents’ impacts on and interactions with the ocean’ – Dr Xu**



These photos illustrate the range of geologic expression for diffuse discharge in seafloor hydrothermal systems: (a-b) the sides of sulfide mounds ‘topped’ by black smokers, (c-d) cracks in the seafloor, and (d) areas of porous seafloor identifiable by bacterial mats. Diffuse flow is also observed from lava tubes. Photos from Ocean Networks Canada and OOI’s Axial Volcano websites.

the environment. For this reason, three researchers from different disciplines have joined forces to find new ways to reveal the dynamics at play at the bottom of the ocean.

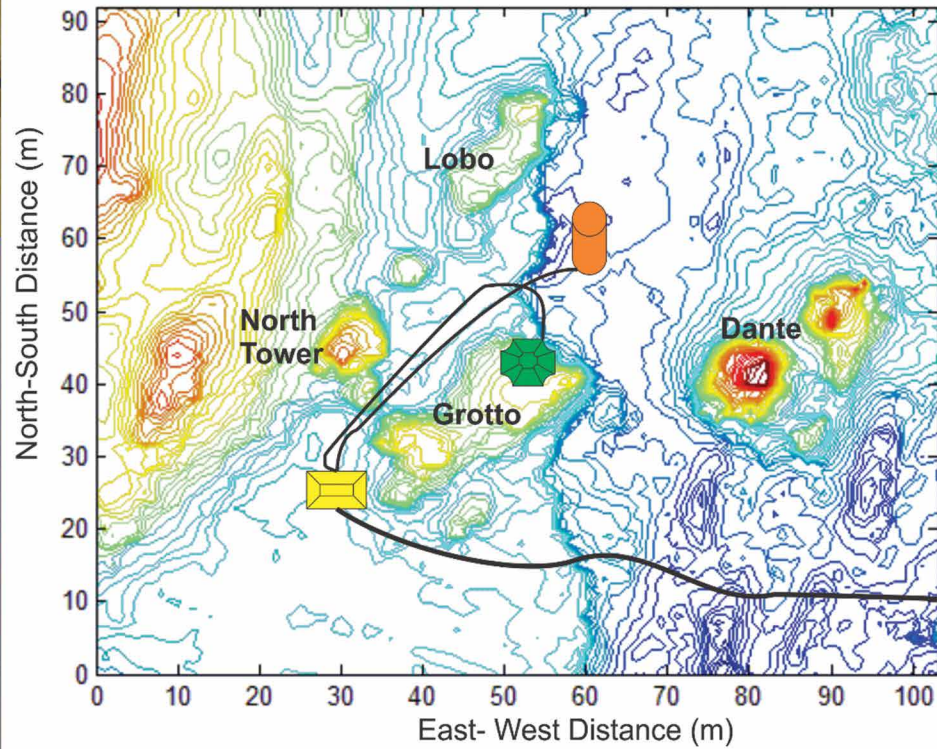
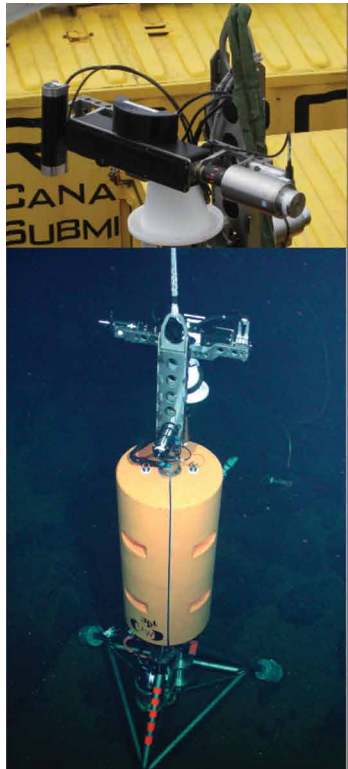
Deep down on the ocean floors, the Earth’s crust is formed at spreading centres, fissures populated with hydrothermal vents. As the name suggests, hydrothermal vents are hot places. There, water gets heated by the geothermal energy of the planet’s interior as a consequence of geological activity at the boundaries of plate tectonics, on mid-ocean ridges. The hydrothermal systems created by water circulating under the sea floor carry heat, chemical substances, microbes, and larvae out of the crust. The vents’ energy

and heat are often exploited by deep sea creatures, who enjoy the microclimate. Often hydrothermal vents are in the form of ‘black smokers’. Black smokers emanate from chimney-shaped structures, formed of iron compounds precipitated as hydrothermal fluid mixes with much colder seawater. One quarter of the total heat lost by the Earth is eliminated through oceanic hydrothermal systems. In addition to black smokers, hydrothermal vents appear as a surprising variety of geological formations, such as flanges, mounds, beehive chimneys, patches and cracks. This activity is very difficult to observe directly due to poor visibility deep down. When it comes to measuring the heat output the buoyant plumes, the abyssal relief

poses new challenges. Three researchers exploring all of these phenomena are Dr Karen Bemis, Dr Darrell Jackson, and Dr Guangyu Xu.

Dr Bemis brings to the collaboration her cross-disciplinary knowledge of computer algorithms and geological phenomena occurring on the sea floor. She is interested in developing computer visualisations of changing geologic phenomena and gaining insight into fluid flow and heat transport in geologic processes. When asked what attracted her to the field, she explained: ‘I decided to study geology because I always liked rocks. As I got older, I became increasingly interested in why rocks differed,





(Left) A close-up of the sonar head on COVIS, mounted 4 m above the seafloor. (Right) COVIS (orange cylinder) is deployed just NE of Grotto in the Main Endeavour Field at depth 2194 m on the Juan de Fuca Ridge and connected to a junction box south of Grotto (yellow trapezoid) as are other instruments sited on the north end of Grotto (green pyramid). Bathymetry from Clague et al., 2008.

how they formed. In college, I focused on the physics of geologic processes. In graduate school, I started studying heat transfer related to hot springs at mid-oceanic ridges. This led me to underwater acoustics as a tool to study plumes of hot water from hot springs on the mid-oceanic ridges. Acoustic imaging has the advantage of both providing a 3D view of the plumes and their surroundings and of leading to quantitative measurements of fluid flow, volume flux and heat transfer.'

Dr Jackson contributes his skills and knowledge of signal processing techniques in underwater acoustics, and notably time-reversed acoustics. He tells us about how the trio came to work on oceanic hydrothermal systems. 'Peter Rona noticed our work on using sonar to measure changes in seafloor temperature. He suggested that this technique could be applied to oceanic hydrothermal systems. Carefully designed sonar measurements can improve understanding of hydrothermal systems, much as ultrasound has improved medicine's ability to observe the human body. Sonar provides a form of remote sensing, replacing a few point measurements with a 3D array of measured values.' Peter Rona was an oceanographer with Rutgers University

who found hot springs on the Mid-Atlantic Ridge in 1985 and is the founder of the collaboration.

Finally, Dr Xu brings his skills in scientific calculation and signal processing to the project. He is highly passionate about the underwater world and having 'the opportunity to explore the many unknowns hidden beneath the surface of the ocean. Every day of my work is filled with the challenge of learning theories and skills and using them to better understand natural phenomena in the ocean. Deep-sea hydrothermal vents are important because they export heat and chemicals to the ocean and support unique benthic ecosystems. Our understanding of the ocean will remain incomplete without fully understanding hydrothermal vents' impacts on and interactions with the ocean.'

#### COVIS is watching

The acoustic imaging of hydrothermal flow first started accidentally in 1984. Back then, a buoyant plume showed up on a routine sonar scan looking for obstacles in the path of ALVIN, a manned submarine. Later on, acoustic data processing and

**'Acoustic imaging has the advantage of both providing a 3D view of the plumes and their surroundings and of leading to quantitative measurements of fluid flow, volume flux and heat transfer' – Dr Bemis**

scientific visualisation were combined into new technologies capable of observing and quantifying what happens during hydrothermal discharges.

Drs Bemis and Jackson started making sonar observations to collect quantitative data on focused and diffuse hydrothermal flows. The project initially involved manned vehicles for deep sea exploration. However, those vehicles only allowed for an hour of exploration at a time. When they employed a Remotely Operated Vehicle (ROV), they could extend their periods of observation to a day in each session. Most recently, they, together with Dr Xu, made observations spanning years in a row by means of a sonar system

connected to the NEPTUNE cabled observatory and operated by Ocean Networks Canada.

The Cabled Observatory Vent Imaging Sonar, or the COVIS, was developed with the help of the National Science Foundation. At its heart is the Reson SeaBat 7125 multi-beam sonar, capable of high-resolution sonar imaging and giving the researchers a way to look into the abyss. In a paper titled 'The path to COVIS: A review of acoustic imaging of hydrothermal flow regimes', the team retraced the steps of scientific discovery from inception all the way to the development of leading-edge technology – the COVIS. Due to it being a cabled observatory residing almost permanently under the water, COVIS has monitored hydrothermal flows eight times a day for several years. Aided by the data the observatory sends back, the researchers were able to develop state-of-the-art diffuse flow detection techniques for estimating plume activity rhythms, bending, vertical rise, and volume and heat flux.

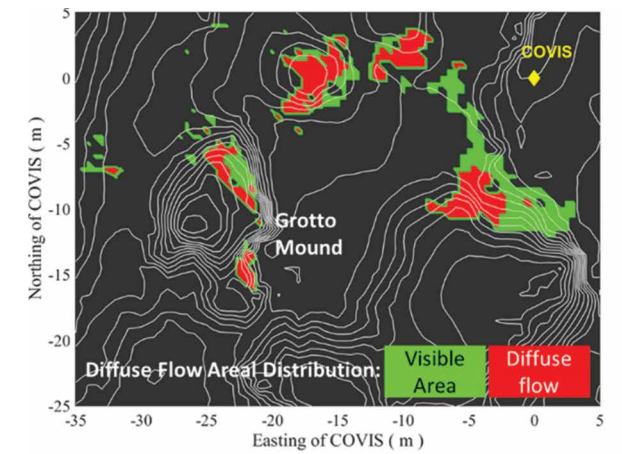
The importance of the research lies in two directions. 'Heat transfer at mid-oceanic ridges is important for understanding the circulation of seawater-based fluids in the context of the plate tectonics system and from a climate perspective, for understanding the heat inputs of the ocean,' Dr Bemis explains.

COVIS' explorations started in 2010. Ever since then, its data have been used to create 3D dynamical images showing the effect of tides on the plumes, velocity measurements, heat transport estimates, and 2D images of spatial distributions of diffuse flows. But COVIS started with the question of whether acoustic imaging can really show the behaviour of the plumes. During the first five years of operations, up until 2015, COVIS performed excellent measurements and brought back a wealth of scientific knowledge. Just like bats, COVIS sees through sound scattering and can take two-dimensional slices through the plumes, thus enabling the reconstruction of 3D images. The sonar can measure the velocity of the flows and shows the presence of diffuse hot water by detecting its shimmering effects like those of a hot roadway. COVIS can also be programmed and controlled remotely during its long missions.

The motion of plumes creates a Doppler shift in the frequency of the sonar echo, which helps determine the velocity of the water current. Together with 3D imaging, this shift helps determine the direction and strength of the current everywhere inside the plume. This is how the team made the striking discovery that the heat production can be larger than 10 megawatts, despite some plumes being thrown out of five holes, each with a size of only five centimetres. Around black smokers, the temperature reaches more than 250 °C, whereas the diffuse part of the plumes has temperatures of only a few tens of degrees. Researchers can see the diffuse flows by pointing the sonar to the ocean floor and programming the computer to listen to its 'pings'. When diffuse flows are present, the sonar echo breaks its normal patterns and the result is transformed into maps of the flows.

#### Future plans and next steps

Cabled ocean observatories started a new era in oceanography. The data they produce reveal the interdependencies between geological, hydrothermal, oceanic, and biological processes. The mid-ocean ridge hydrothermal systems are especially interesting because of the larger implications they have for ocean ecosystems and climate.



The incidence (red) of decorrelation intensity above an arbitrary threshold is shown for those locations visible (green) to COVIS on a bathymetric map of Grotto mound. Areas of high decorrelation (red) are generally located in areas geologically likely to have diffuse discharge. COVIS's location is shown on the map for reference (yellow diamond).



**'Sonar provides a form of remote sensing, replacing a few point measurements with a 3D array of measured values' – Dr Jackson**

The plans for this project are only beginning, now that the data have confirmed the possibility of investigating the hydrothermal flux by acoustic imaging. 'We want to continue and expand this work into the US cabled observatory in the Eastern Pacific. Also, we hope to develop similar techniques for use on mobile platforms, ships and remotely-operated vehicles,' Dr Jackson tells us. Dr Bemis adds that 'having developed the COVIS and gained a great deal of understanding of plumes and the mechanisms of acoustic scattering by plumes, the next step is to develop an exploratory tool that would allow AUVs (autonomous underwater vehicles) and ships to identify plumes sourced from hot springs on mid-oceanic ridges as they survey the ocean floor'. Being a marine scientist, Dr Xu is interested in 'exploring the far-reaching impacts of a deep-sea hydrothermal vent system on the ambient ocean. The targeting questions will be how the geochemical and biological matters are released from a hydrothermal vent site dispersed within the deep ocean and how far away from the vent site can they reach. How are those dispersal patterns driven by the ocean currents?'



# Meet the researchers



**Dr Karen Bemis**

Department of Marine and Coastal Sciences  
Department of Earth and  
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Rutgers University  
USA

Dr Karen Bemis received her PhD in Geological Sciences at Rutgers in 1995 with work on morphology of volcanoes, especially small explosive volcanoes called cinder cones. In 1996, she continued to work with Peter Rona on hydrothermal systems as a postdoc at Rutgers where she was introduced to Deborah Silver and scientific visualization. Currently, she's a Research Associate in the Department of Marine and Coastal Science at Rutgers. She continues to study cinder cones in Guatemala and sonar imaging of hydrothermal systems.

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**Dr Darrell Jackson**

Applied Physics Laboratory  
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Dr Darrell Jackson has two PhDs, one in electrical engineering and a second one in physics, from California Institute of Technology. He is a researcher and Research Professor Emeritus with the University of Washington Department of Electrical Engineering and a member of the Acoustical Society of America. His current research is focused on the application of multi-beam sonar to two different problems in acoustical oceanography: classification of the seafloor and observation of undersea hydrothermal vents. Past research has included signal processing techniques in underwater acoustics.

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**Dr Guangyu Xu**

Woods Hole Oceanographic Institution  
USA

Dr Guangyu Xu received his PhD in 2015 from Rutgers University is currently a researcher in marine sciences at the Marine Geology & Geophysics department of Woods Hole Oceanographic Institution. His research interests include the dynamics of deep-sea hydrothermal plumes and their interaction with ambient deep-sea currents and underlying geological processes. He already published a large number of research papers, held talks at multiple conferences and was engaged in teaching activities. He is proficient in scientific calculation and signal processing and enjoys fiction reading, swimming, and scuba diving in his little spare time remaining after his research activities.

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## FUNDING

US National Science Foundation



## UNCOVERING THE MYSTERIES OF MARINE MERCURY

Mercury levels, primarily as methylmercury, in fish and seafood are a global concern. Marine scientist, **Professor Robert Mason** of the University of Connecticut in the USA, studies how mercury levels in the biosphere are changing due to climate change. He has collaborated internationally to study mercury levels in the ocean's surface and the lower atmosphere. One focus of his work is currently on mercury levels in the Arctic Ocean, where climate change effects are most pronounced.

### What is Mercury?

Mercury is a trace metal, meaning it is generally present at low levels in the environment. While it has natural sources to the atmosphere, it is also released during energy production, primarily from coal burning, and other industrial activities. It has also been used in many industrial applications. For example, once the fluid commonly found in thermometers, its toxicity has led to mercury thermometers becoming instruments of the past, phased-out in favour of less harmful ethanol thermometers. Professor Mason at the University of Connecticut explains what attracted him to the study of this element: 'It is a fascinating metal with complex chemistry: liquid at room temperature and a gas in the air; toxic and bioaccumulative; and human activities have substantially exacerbated its concentration in the biosphere.'

### Mercury's Biogeochemical Cycle

This increase in mercury concentration in the global environment is what Professor Mason's research focuses on. Alongside his colleagues, he seeks to understand how mercury is transported and transformed in the atmosphere and in aquatic systems, to ultimately find out where it ends up, and how it accumulates as methylmercury in seafood. In their work, the team studies the open ocean, coastal zones and freshwater systems, in addition to the processes occurring in water, air, sediment, and at their interfaces. This knowledge will allow Professor Mason and his students, post-docs and collaborators to better understand the bioavailability and bioaccumulation of mercury into biota in aquatic systems. Professor Mason describes how mercury moves around the biosphere: 'Mercury travels



through the atmosphere mostly as a gas in its elemental form ( $\text{Hg}(0)$ ), and is transported globally from its sources, both natural and anthropogenic, prior to being deposited back to the terrestrial and ocean surface mostly as ionic mercury ( $\text{Hg}(\text{II})$ ). Chemical and biological processes in soils, vegetation and the ocean can either convert the  $\text{Hg}(\text{II})$  back to  $\text{Hg}(0)$ , so it can be released again to the atmosphere and transported further, or transform it to methylmercury ( $\text{MeHg}$ ). The conversion of  $\text{Hg}(\text{II})$  to  $\text{Hg}(0)$  therefore impacts the amount transformed into the more toxic and bioaccumulative  $\text{MeHg}$ . A current focus of Professor Mason's work is on the mercury water-air exchange fluxes in the Arctic Ocean, where the effects of climate change are most severe. 'Mercury enters the Arctic Ocean surface waters from



## ‘Understanding mercury exchange at the sea surface – how much is released back to the atmosphere – is fundamental to understanding the impact of human activity on methylmercury concentrations in ocean fish and seafood consumed by humans and wildlife’



the atmosphere, rivers, erosion, ice melt etc., mostly as Hg(II),’ Professor Mason tells us, when asked about the mercury cycle. ‘In surface waters it is reduced to Hg(0) and this form of mercury is a dissolved gas in water. It is volatile, so can be lost to the atmosphere. In the atmosphere, chemistry converts the Hg(0) to Hg(II), and the cycling repeats. Ice cover mediates the extent to which this exchange occurs.’

### Mercury Bioaccumulation

Professor Mason elaborates on why the build-up of mercury, in the oceans and subsequently as MeHg in organisms, is of concern: ‘Some of the mercury entering the ocean is converted to MeHg, whose elevated concentrations in seafood and freshwater fish are a global health concern.’ Most human exposure to the MeHg neurotoxin, comes from our consumption of large predatory fish, such as tuna. He adds: ‘About 90% of the mercury entering the ocean from the atmosphere – with precipitation, and particle and gas deposition – and via rivers, point source inputs and groundwater, is lost back to the atmosphere due to the conversion of the Hg(II) back to Hg(0) and

subsequent gas evasion. If this was not the case, the contamination of the ocean by human activity would be much greater than it actually is. Understanding this exchange at the sea surface – how much is released back to the atmosphere – is fundamental to understanding the impact of human activity on MeHg concentrations in ocean fish and seafood consumed by humans and wildlife.’

### Atlantic Adventure

Between 2008 and 2010, Professor Mason and his team participated in six research cruises in the West Atlantic Ocean, which were funded by the U.S. National Science Foundation (NSF), Chemical Oceanography division. Two of these departed from the east coast of the United States, and four were conducted in the waters surrounding Bermuda. During the expeditions, the team simultaneously measured the concentration of both sea surface and atmospheric mercury at high resolution, together with the total mercury distribution. They found that Hg(0) levels were lowest near coastlines and regions influenced by river inputs, while higher levels were found in the open ocean. They also found that levels of Hg(0) varied by

more than a factor of three between cruises. The team concluded that they needed more information on the role of dissolved organic carbon in the ocean, and how this affects the redox kinetics of Hg(0) and Hg(II) in the marine environment. With this, they would then be able to improve their estimates of mercury exchange between the sea and the air.

### Pacific Pursuits

Then in October 2011, Professor Mason’s group was involved in an additional NSF-funded expedition into the Pacific Ocean, in the waters between Hawaii and Samoa. The team set out to further understand the variability of mercury quantities in both the ocean and atmosphere. To do this, they again collected high-resolution measurements of mercury spanning large gradients in seawater temperature, salinity, and productivity. Their measurements were then input into an ocean general circulation model coupled with an atmospheric chemical transport model, in order to create a model for the mercury inputs and losses in the surface ocean.

### High Mercury Levels at the Intertropical Convergence Zone

The research group found greater variability in the amount of Hg(0) in the sea surface water than in the surrounding atmosphere. Additionally, concentrations of Hg(0) at the intertropical convergence zone were three times higher than in surrounding regions. This agreed well with observations from the Atlantic Ocean expeditions. The models created by Mason’s collaborators at Harvard University revealed that the high levels of surface ocean Hg(0) in this zone are due to a combination of high precipitation levels and a shallow surface ocean mixed layer. However, the model underestimated the amount of mercury in the surface ocean, likely due to the model’s incomplete parameterisation of scavenging of reactive mercury ions in the upper atmosphere. While the highest concentrations of Hg(0) were found in the ITCZ, the highest MeHg concentrations in zooplankton were near the equator, indicating differences in the locations of Hg(0) formation and that of MeHg bioaccumulation. These differences point to the complexity of how changes in Hg inputs and Hg(0) evasion impact the accumulation of MeHg in the food chain.



Sofi ice sampling in the Arctic. CREDIT: Michelle Nerentorp Mastromonaco

### Arctic Exploration

These expeditions to the Atlantic and Pacific Oceans led Professor Mason and his team to propose studies in the Arctic Ocean as part of the international GEOTRACES Program. This program is an international effort to examine the distributions of trace elements and isotopes in all the ocean’s waters. This latest cruise departed from Alaska aboard the US Coast Guard Research Vessel *Healy*, taking a route north through the ocean and sea ice, to the North Pole and back, between August and October in 2015. ‘The Arctic Ocean is an ocean that is responding most dramatically to temperature rise and climate change. Therefore, studies in the Arctic Ocean can help us understand the combined potential impacts of changes in human emissions of mercury, which are now dominated by anthropogenic emissions from Asia, and how climate change will affect levels of methylmercury in seafood and therefore human exposure,’ Professor Mason tells us. The measurement approach throughout the cruise was similar to those taken in the previous studies and were made continuously throughout the trip. The ship initially sailed through open water, and then through increasing amounts of ice cover, from marginal to total ice cover. During their return, the opposite trend was seen.

### Arctic Ice and Its Effects on Mercury Levels

Although many of the results from the expedition are still under analysis, some interesting findings have already been released. Professor Mason talks about his observations so far: ‘Based on the measured concentrations of Hg(0) in the surface waters and in the atmosphere, it is possible to estimate the potential rate at which Hg(0) could be lost to the atmosphere. Of course, under ice this is a potential flux, and not a real flux. As the cruise travelled to the pole and back, we saw increasing potential fluxes as ice increased, and then it decreased in the latter part of the cruise as ice cover was again reduced. In open waters there was little flux.’ He adds: ‘We can use differences in the concentrations in the water and typical conditions of wind and temperature to estimate how long it would take the built-up Hg(0) to be lost, once ice is removed. Our initial findings suggest it would take several months. Also, we can compare these potential flux rates to other ocean regions.’

### The Mercury Cycle in the Arctic

Prior measurements and models suggest that approximately 60% of the Hg(II) added to the surface waters of the Arctic is converted into

Hg(0), and 90% of that is then lost to the atmosphere, and the group’s recent results agree with these estimates. More Hg(0) appears to return to the atmosphere than is deposited from the atmosphere to the sea. In addition, given the team’s findings, the extent of loss of Hg(0) to the atmosphere will depend on the amount of ice and its persistence in summer. Hg(0) builds up to much higher concentrations under the ice than in the open water. When the ice melts, this will cause a large, short-term release of Hg(0) to the atmosphere over several months. Thus, the extent and duration of ice cover effects the amount, and rate of release, of Hg(0) into the atmosphere. When more Hg(0) is lost to the atmosphere, through a complex set of interactions, this leads to less Hg(II) being transformed into methylmercury and therefore lower amounts of its subsequent accumulation in fish and other sea creatures.

### Methylmercury and Dimethylmercury in the Arctic

The remaining Hg(II) added to the sea is transported downward and mixes into the deeper waters of the ocean. Here, it is methylated into toxic and bioaccumulative methylmercury. It is then also converted into toxic dimethylmercury, which can evade back into the atmosphere. Sofi Jonsson, a post-doc with Mason, recently returned from another Arctic expedition on the Swedish Research Vessel *Oden* in collaboration with Swedish colleagues, where she collected samples for MeHg and total Hg analysis, measured dimethylmercury on board, and did experiments examining the formation and degradation of MeHg in Arctic waters. These results build on studies of photochemical degradation of MeHg being done by Mason’s graduate student, Brian DiMento, and will aid in understanding the extent to which dimethylmercury formed is lost to the atmosphere, and where its major formation occurs. Recent studies completed by Sofi and graduate student, Nash Mazrui have suggested that dimethylmercury formation could occur through the reactions of MeHg with sulfide minerals and organic matter in ocean waters. As the ice and permafrost is predicted to melt significantly as a consequence of climate change in the coming years, the team express concerns that this may lead to potentially higher levels of methylmercury in the Arctic food chain. However, the current results suggest that this could be offset by a potential decline in mercury concentrations driven by increased loss to the atmosphere in ice-free surface waters. Results of samples currently being analysed will lead to a better understanding of the rate of loss of mercury from the ocean as Hg(0) or dimethylmercury, and what then happens to it. Finally, global and regional regulatory actions, such as those proposed under the United Nations Minamata Convention on Mercury, that decrease Hg inputs to the ocean should also rapidly affect surface water aquatic mercury concentrations, even in the Arctic which is remote from these anthropogenic inputs.

### Further Studies into the Role of Microorganisms on Methylmercury Levels

When asked what lies ahead for his team’s research, Professor Mason concludes by saying: ‘The chemistry of mercury in the ocean and the role of microorganisms in transformations of Hg(II) into MeHg, and also the processes of formation of dimethylmercury, are all focuses of my currently funded research, along with the studies examining the inputs and outputs of mercury from ocean systems, both the open ocean and coastal environments.’





# Meet the researcher

**Professor Robert Mason**  
Professor of Marine Sciences and Chemistry  
Department of Marine Sciences  
University of Connecticut , USA

Robert Mason is Professor of both Marine Sciences and Chemistry at the University of Connecticut in the USA. Professor Mason completed his BS and MS degrees in chemistry in his native South Africa, and then went on to work at the federal South African Sea Fisheries Research Institute, before moving to the USA to study for his PhD in Marine Science at the University of Connecticut. Following its completion in 1991, he went on to a post-doctoral position at MIT and then to the University of Maryland, Center for Environmental Sciences prior to returning to the University of Connecticut to take up his current position.

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Celia Chen, Dartmouth College

## FUNDING

NSF/Chemical Oceanography Division  
NSF/CHEM  
NIH/NIEHS  
NOAA/Sea Grant

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# MARINE MICROBES SHED LIGHT ON OUR CHANGING OCEANS

Climate change and anthropic interactions with the oceans are making marine habitats increasingly unsuitable for marine animal life. This is why researchers like **Professor Gordon Taylor** at Stony Brook University are working to understand the complex dynamics governing water chemistry through studying microbial life.

## Gazing into the planet's past

Virtually every modern animal living on Earth today requires free oxygen to complete its respiratory metabolism – but this has not always been the case for all organisms in our evolutionary past. The planet's ancient history was dominated by oceanic waters devoid of dissolved oxygen, where only microbial life could thrive. As time passed, most marine habitats acquired higher levels of oxygen, as a direct result of photosynthetic microorganisms splitting water molecules and releasing free oxygen. Microscopic life forms living in the oceans are an integral part of the chemical and biological cycles of the areas they inhabit by exchanging chemicals with the environment or using the chemicals present in their surroundings.

In order to understand the ocean ecosystems and their health, Professor Gordon Taylor's research explores the way in which microbes use and exchange chemicals with the environment, with a focus on what happens at the interfaces between habitats. Interfaces are the boundaries between different biotic regimes, places where alien worlds come in contact with each other. In many regions, oxygenated oceanic waters gradually make way for waters with lower oxygen concentration at depth called hypoxic waters, and those depths with virtually no oxygen content are called anoxic waters. The Cariaco Basin, a veritable window into the planet's past, is the largest oceanic habitat in the world that is permanently anoxic. This basin is the target of Professor Taylor's investigations due to its uniqueness and its potential to offer data regarding how microbes mediate the cycling of elements

essential for life on Earth in large amounts, such as carbon, nitrogen and sulphur.

## Portrait of an oceanographer

Today, Professor Taylor is a microbiological oceanographer at Stony Brook University specialising in biogeochemistry, microbial ecology, hypoxic and anoxic regimes, processes occurring at interfaces between marine habitats, and climate change. Throughout his career, his research has been funded multiple times by the National Science Foundation, which also recently enabled him to found the NAno-RAMAN Molecular Imaging Laboratory (NARMIL) in 2014. The Confocal Raman Microspectrometer and Atomic Force Microscope within this facility provide the means for his group to better understand the biogeochemical roles of specific microorganisms cell-by-cell. (visit: <http://you.stonybrook.edu/nanoraman/>). Furthermore, the equipment in this lab supports a wide variety of research in the fields of marine, atmospheric, environmental, biological, chemical and materials sciences. Professor Taylor is an honoured member of the Association for the Sciences of Limnology and Oceanography, and a member of the American Society for Microbiology, the American Geophysical Union, and the International Society for Microbial Ecology. He is on the editorial board of four important journals including *Limnology & Oceanography: Methods* and is an active reviewer for 32 other scientific publications and 14 domestic and international funding agencies. Among his professorial activities, he has served on the doctoral and master's thesis committees of more than 70 students



*M. Pachiadaki and V. Edgcomb preparing submersible sampler developed at Woods Hole Oceanographic Institution that preserves gene expression samples in situ.*

and taught courses in oceanography and marine microbiology. Professor Taylor has participated in more than 80 research cruises and expeditions and led 37 cruises himself. The most recent cruises centred on Three Domain MetaOmics Study of the Cariaco Basin, in Venezuela.

Professor Taylor tells us what motivated him to pursue a career in this field. 'Ever since childhood, I've always been curious about how things work and how various components interrelate, whether they were mechanical objects or in the natural world. I whiled away my youth taking machines apart and reassembling them, building playthings



**‘In aquatic systems, adequate concentrations of dissolved oxygen gas are essential for the respiration and vitality of all animals and most plants. A growing body of evidence suggests that concentrations of dissolved oxygen are declining across broad regions of the global ocean, resulting from climate change – so-called ocean deoxygenation.’**



*Venezuelan research vessel supporting the Cariaco Basin Ocean Time Series Program*

from scrap yard junk, or making interesting and sometimes dangerous concoctions with my chemistry set,’ he explains. ‘But my real passion was to comb the local fields, forests, riverbanks, and lake shores of rural upstate New York for reptiles and amphibians that I could take home as pets. I kept detailed notebooks on these animals and their Mesozoic predecessors.’

By the end of his undergraduate studies, marine ecosystems held Professor Taylor in their thrall. ‘I wanted to know everything about them and how they supported such diverse and fascinating life forms,’ he tells us. ‘When I began my graduate studies, microbiological oceanography was in its infancy with so many unanswered questions and challenges. The most basic and foremost of these challenges was and still is identifying marine microorganisms and their respective ecological roles. For example, to this day the identities and roles

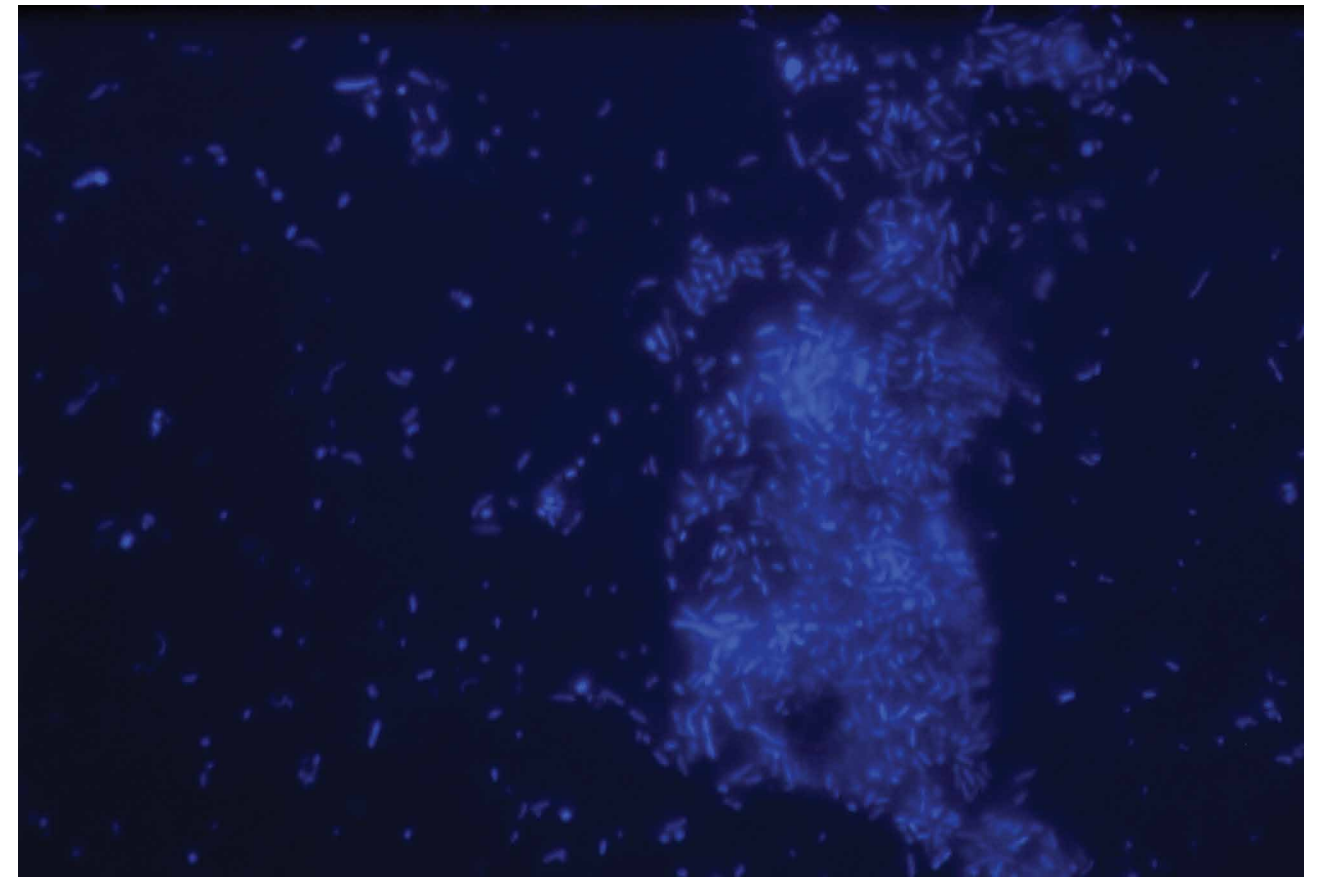
of more than 90% of all marine microbes are unknown, largely because most species elude laboratory study by refusing to grow in culture. Advances in genetic sequencing over the last two decades, however, have somewhat diminished the microbiologists’ cultivation imperative and catapulted the field forward. In the early 1990s, my lab studied the role of marine microbes in generating seasonal hypoxia-anoxia in the nearby Long Island Sound. This led to my involvement in a project to study the world’s largest permanently anoxic marine basin, the Cariaco Basin, with my colleague, Professor Mary Scranton. One of many reasons that this system is fascinating to me is because it offers a window into Earth’s ancient past when the entire ocean was anoxic and all life was microbial. This ocean biogeochemical time-series program continues to be a major research focus for us. We have explored time and depth-dependent variations in water column structure and in chemical

and microbiological processes for the last 21 years. To better understand variations in community composition and capabilities and ecosystem function, we have teamed up with several molecular microbiologists over the years.’

#### **Marine habitat diversity and climate change**

Typical marine water columns are divided into multiple layers, each with its own biological community adapted to the surrounding chemistry and oxygen levels. Global warming is causing major changes in these habitats by amplifying the density differences between layers, decreasing the penetration depth of oxygen, and affecting the solubility of gases in water. Consequently, scientists have noticed that the regions depleted of oxygen are expanding, and therefore the ocean’s global balance of carbon, oxygen, nitrogen and sulphur is shifting. As new nutrients pour into the coastal ocean from the land – a phenomenon known as eutrophication – the so-called dead zones expand throughout nearshore regions and estuaries, impacting fauna occupying these waters and the sea floor. We should recall that this is the case because oxygen levels generally decrease with depth, and depending on the affected region, the intensity and duration of oxygen depletion varies from seasonal hypoxia to permanent anoxia. Eutrophication leads to the proliferation of algae, some of which produce harmful toxins. Moreover, excessive algal populations promote oxygen depletion. ‘In extreme cases, this chain of events leads to anoxic waters that may produce hydrogen sulfide which is toxic to most animals,’ Professor Taylor warns. Lack of oxygen leads to an entire chain of consequences. Aside from being harmful to animal life, it impacts the way that essential minerals are cycled. An example provided by Professor Taylor is that ‘loss of biologically available nitrogen is accelerated in oxygen minimum zones (OMZs) found along the eastern margins of the Pacific, Atlantic and Indian Oceans and other oxygen-depleted bodies of water. Without a compensating mechanism to replenish bioavailable nitrogen, expanding zones of oxygen depletion imply less nutrition to support plankton production in future oceans.’

This is one of Professor Taylor’s reasons for studying the Cariaco Basin. The basin acts as a natural laboratory, allowing researchers to gain insight into the microbiological and



*Epifluorescent microscope image of DAPI-stained cells from the oxic-anoxic interface during a Cariaco time-series cruise (12 May 2014). Image shows Bacteria and Archaea organised around a particle. Magnification: 1000X. (Photo credit: E.A. Suter).*

biogeochemical processes in the transition zones between oxygenated and deoxygenated waters. Conveniently, it is a single system where the whole range of oxygen conditions are available for study. ‘What we learn here informs us about how oxygen-depleted water columns elsewhere currently function and how they might function in the future as deoxygenation progresses,’ he explains. ‘Furthermore, the CARIACO Ocean Time-series program has provided monthly data on physical, chemical, biological and meteorological conditions since late 1995. This outstanding database provides a unique and very important context to facilitate understanding our molecular results on community composition, functional genes present and gene expression.’

Despite the wealth of knowledge researchers have already gathered, fundamental questions still persist about life forms feeding on inorganic and organic chemicals and living without oxygen, especially how they cycle nutrients present in the water. Although DNA and RNA sequencing technologies have enabled scientists to acquire an understanding of the genetic diversity and classification of microbes in nature, these technologies do not adequately capture the functional dynamics of microbial communities present in any given environment. Thus, there is more to learn about potential and realized capabilities of marine microbes. Their functional diversity and redundancy and their responses to chemical and physical changes in their ecosystems are still poorly understood. A particularly interesting part of Professor Taylor’s research aims to find out how microbes adapt to oxygen depletion by studying their behaviour in the context of geochemical gradients in their environment. These gradients represent distributions of chemical species in the water and are influenced by depth, currents

and other local characteristics of the medium. In order to feed, microbes organize along these gradients and, through time, modify their metabolism and gene expression to better adapt to current conditions.

#### **Future research directions**

Since most marine microbes to date cannot be cultivated in the lab, scientists need ways to explore their functions without the benefit of laboratory cultures. DNA and RNA inventories of microbial communities tell us about the genes present and whether they are being used at the time of collection. These inventories are powerful tools for gaining a better understanding of metabolic pathways and the response of microbial communities to environmental conditions. Previous experience and the database amassed during Professor Taylor’s research, has enabled his team to make important steps towards developing techniques capable of linking key players to particular biogeochemical functions and activities of microbes. ‘We do so by allowing active members to incorporate stable isotopic tracers of a process and produce isotopically heavy biomass and we fluorescently-label subsamples with a selection of genetic probes. We then interrogate individual probe-positive cells in each taxonomic subsample by confocal Raman microspectrometry to determine extent of isotope incorporation. These single-cell analyses are performed in the NARMIL.’ Professor Taylor tells us. Among many other types of research, the lab enables scientists to determine which organisms dominate carbon, nitrogen and sulfur cycling in anoxic systems and to explore elemental cycling within anaerobic symbiotic associations.





# Meet the researcher

**Professor Gordon Taylor**  
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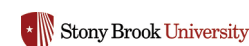
Professor Gordon Taylor at the Stony Brook School of Marine and Atmospheric Sciences has been researching oxygen depleted marine habitats for more than 23 years. After receiving his PhD from University of Southern California, Los Angeles in 1983, he has held several positions with both the University of Hawaii and Stony Brook University, where he became a full professor in 2005. In 2014, he founded the NANO-Raman Molecular Imaging Laboratory at Stony Brook through a National Science Foundation grant. There, he continues his research through his research project on 'Genetic and Metabolic Signatures of Marine Microorganisms in Oxygen Depleted and Varying Geochemical Seascapes'.

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## FUNDING

NSF OCE-1335436 (V. P. Edgcomb, GT Taylor, C Taylor, co-PIs)  
NSF OCE-1259110 (M Scranton, G Taylor, co-PIs)  
NSF OCE-1336724 (G Taylor & D Knopf, co-PIs)  
Gordon and Betty Moore Foundation, Grant #5064 (GT Taylor, PI; J Martínez Martínez & VP Edgcomb, collaborators)

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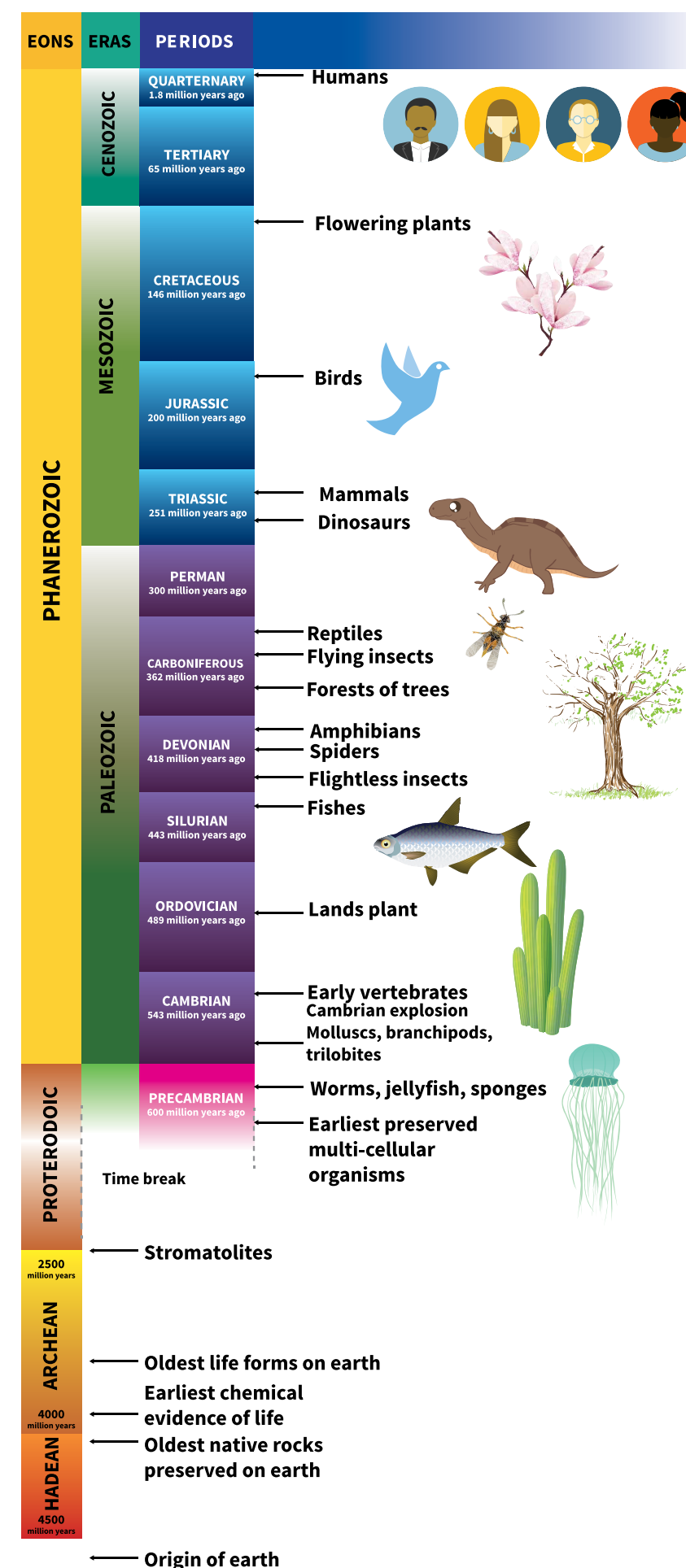
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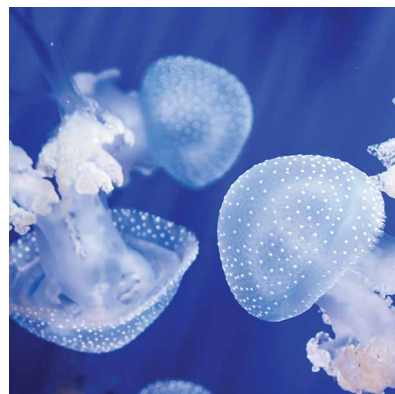
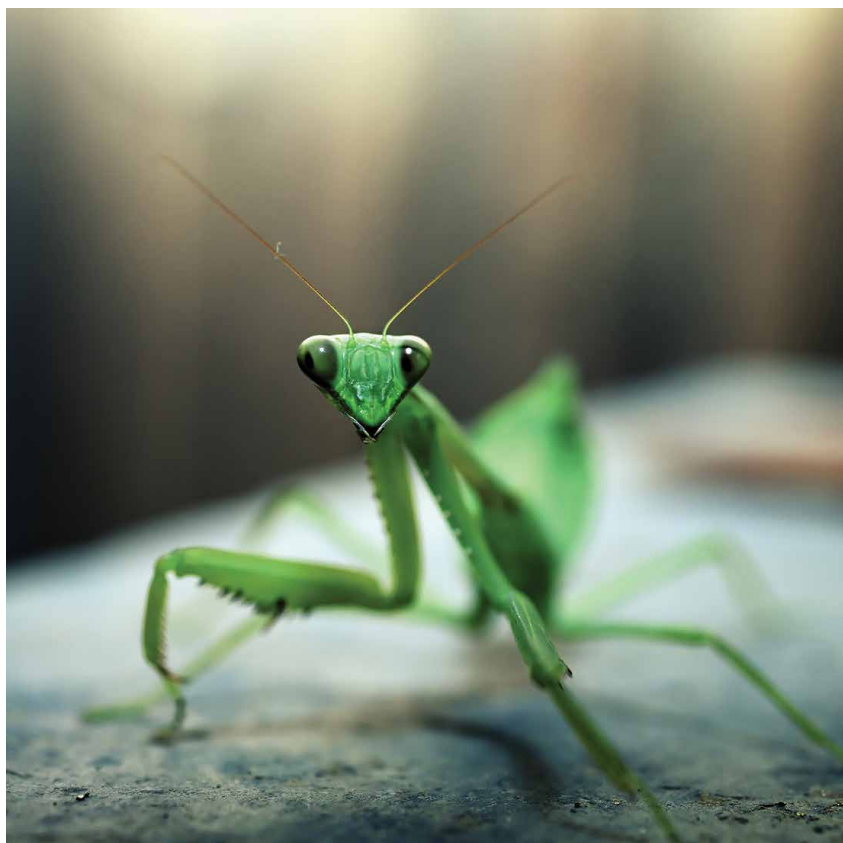


# THE EVOLUTION OF LIFE ON EARTH

Detailed over the last few pages, Professor Gordon Taylor's discoveries in the field of marine microbiology have given us an intriguing picture of what our planet's ancient past was like, when the entire ocean was completely devoid of oxygen and all life was microbial. Although we still don't know exactly how the first life forms came into being, most scientists agree that unicellular organisms appeared in the oceans between 3.8 and 4.1 billion years ago, under the extreme conditions that plagued the planet soon after its formation. The diversification of these simple life forms through genetic mutations into the estimated 8.7 million species that inhabit the planet today is the theme of our next section of the magazine, where we explore the evolution of life on earth.

Continuing our investigations into unicellular life, we introduce Dr Dilianna Simeonova and her colleagues at the Bulgarian Academy of Sciences, who explore how bacteria transform phosphorus compounds to gain energy, survive and proliferate in extreme environments. From these prokaryotes, we move on to the eukaryotes – organisms whose cells possess membrane bound organelles, such as the nucleus. Here, we showcase the research of Dr Bettina Sonntag and her team at the Research Institute for Limnology in Mondsee, Austria, who explore the lives of ciliates – unicellular organisms that live in freshwater lakes. As part of their investigations, the team looked at symbiotic relationships that have evolved between certain ciliate and algal species, and uncovered how algae help to protect these ciliates from harmful UV radiation.





Next, we make the transition to multicellular life, where we showcase two research projects focused on the humble mollusc. Here we introduce Professor Stephen Goff and his colleagues at Columbia University, who study how shellfish – such as clams and mussels – can develop cancer. Although expected to have a viral origin, the team discovered that the disease is directly transmissible from shellfish to shellfish through contagious cancer cells. Also possessing a keen interest in molluscs is Professor Daniel Jackson at the University of Göttingen, who uses snails to study how animals fabricate biominerals. The evolution of this ability, more than 540 million years ago, allowed early animals to precipitate calcium and other minerals in highly controlled and useful ways, leading to the rapid and widespread diversification of animal life. From here, we feature the work of Dr Craig Albertson at the University of Massachusetts, who also investigates how animals have evolved hard parts. Using the craniofacial skeleton in bony fishes as his main experimental model, Dr Albertson's findings suggest that while genes play a critical role in generating variation, nearly every morphological trait he examined was highly influenced by the environment. Keeping with the theme of evolution in fish, next we highlight the research of Professor Arthur DeVries of the University of Illinois –

the discoverer of antifreeze proteins in polar fishes. These proteins enable polar fish to survive and thrive in polar environments, where they would otherwise freeze to death. This unique adaptation forms a cornerstone of the polar ecosystem and biodiversity, as a host of other species are reliant on these fishes as a food source.

Moving on, we jump out of the ocean and arrive high in the mountains of Eastern California, to study a beetle living on the edge of extinction. Here, we meet Professors Nathan Rank, Elizabeth Dahlhoff and their students, who explore the question of evolutionary responses to changes in climate by investigating the Sierra willow leaf beetle – *Chrysomela aeneicollis*. The team have identified several genes that are involved in the beetle's response to stress and have quantified their effects on survival and reproduction. Amazingly, their findings show that evolution is going on right before our eyes in this species of beetle, as it adapts to our changing climate.

Next, we move on to study our feathered friends, by highlighting the work of Professor Elizabeth Adkins-Regan of Cornell University, who investigates social and reproductive behaviour in birds. Specifically, Professor Adkins-Regan's group explore the physiological mechanisms that have

evolved to give rise to life-long bonding in monogamous pairs, parental behaviour and the influence of parents on offspring behaviour. Continuing with the theme of animal behaviour, we showcase the research and conservation work of Professor John Vucetich at Michigan Technological University. Professor Vucetich spends much of his research life studying wolves and moose in Isle Royale and Yellowstone National Parks. In addition to his ecological research, he is also deeply involved with carnivore conservation throughout North America – work that depends on the successful marriage of scientific and ethical knowledge.

Finally, we close this section of the magazine with the study of our own species, *Homo sapiens*. Here, we detail the work of Professors William Hunt and Ralph Hartley at University of Nebraska-Lincoln, who study mysterious human-built piles of rock called 'cairns' scattered over the coastal mountains of Alaska. Before this pair of anthropologists had started researching cairns in this region, the common academic belief was that high-mountain cairns were rare and relatively unimportant. The team disproved this common consensus, by discovering an abundance of these baffling structures in the region and measuring their age through the use of radiocarbon dating and lichenometry.

## DECIPHERING UNKNOWN AND UNRECOGNISED PHOSPHORUS-MICROBIAL TRANSFORMATIONS

Dr Diliana D. Simeonova and her colleagues at the Bulgarian Academy of Sciences, Institute of Microbiology in Sofia, Bulgaria perform research at the interface between geochemistry and microbiology – or simply geomicrobiology. Dr Simeonova and her colleagues are specifically interested the ability of bacteria to transform different inorganic compounds and elements in so many different ways, to gain energy, to survive and proliferate in extreme environments.

### The importance of phosphorus

Gaining an understanding of the basic building blocks of life is an important goal in biology and chemistry. Carbon, hydrogen, nitrogen, oxygen, phosphorus and sulfur are considered the six essential elements of life. Among these, phosphorus is important for the nutrition and metabolism of living organisms. Phosphorus is widely available in the natural environment, and it's estimated that its abundance in the Earth's crust is approximately 0.12% (w/w). This element is found in various forms in nature, including mineral forms, such as apatites, organic forms such as in phospholipids, nucleic acids and proteins, dissolved inorganic forms in various oxidation states (P[+V], P[+III], P[+I] species), and gaseous forms in the [-III] oxidation state. In a soluble form, it is most frequently found at a neutral pH as orthophosphates ( $\text{H}_2\text{PO}_4^-$  and  $\text{HPO}_4^{2-}$ ), and the predominant form of phosphonic acid at a neutral pH, is the hydrogen phosphite ion ( $\text{HPO}_3^{2-}$ ) or phosphonate, containing phosphorus in the [+III] oxidation state. Phosphonic acid compounds are divided into two classes: organophosphonates (phosphonates), which are characterised by a direct C-P bond, and inorganic phosphonates (phosphites). Furthermore, phosphonates are separated into those that occur naturally and

those of an anthropogenic origin.

A wide variety of reduced inorganic phosphorus compounds of anthropogenic origin have multiple uses, including phosphorus triiodide ( $\text{PI}_3$ ), which is a reducing agent used in organic chemistry in the conversion of alcohols to alkyl iodides, aluminium phosphide (ALP), which functions as a semiconductor, a fumigant, a rodenticide or an insecticide, and phosphorus trichloride ( $\text{PCl}_3$ ), which is the precursor of many organophosphonates with an anthropogenic origin. Currently, inorganic phosphites enter the environment because of human activities, mainly as pesticides, insecticides and herbicides. Since the 1970s, the use of phosphite-based fungicides, against *Oomycota* plant-pathogens of the genus *Phytophthora* in agriculture has increased. In addition, some phosphite-containing formulations are offered and used in agriculture as fertilisers, which increases the controversy surrounding these compounds.

Based on the above knowledge of phosphorus, researchers have revealed that reduced inorganic phosphorus compounds serve as a phosphorus source in the assimilatory metabolism of aerobic bacteria and in anaerobic bacteria. However,

the utilisation of the reduced inorganic phosphorus sources as electron donors in the energy metabolism of bacteria is still not fully understood. As Dr Diliana D. Simeonova explains: 'there are many white patches in our knowledge about this element, its cycle in nature, interactions, and so on. Therefore, I find it impressive to work in this domain, to contribute to the deciphering of the unknown and unrecognised phosphorus-microbial transformations.' Thus, the studies Dr Simeonova and her colleagues focus on the genes and their products that are involved in the process of anaerobic dissimilatory phosphite oxidation, which occurs in the bacteria species called *D. phosphitoxidans*. 'Bacteria are ubiquitous, there are no habitats without bacterial presence,' Dr Simeonova tells us. 'Thus, by studying these processes, one may contribute to the better management of phosphorus and phosphorus sources, considering this is one of the five major vital elements, indispensable for life.'

### 'Unknown Genome' Proteomics

The genome of *D. phosphitoxidans* had not yet been sequenced at the time Dr Simeonova's started this research, and thus, her team developed a new approach to access genes and their products in this bacterium. Basically, they started with



‘Of huge interest to me is the ability of bacteria to transform different inorganic compounds and elements in so many different ways, to gain energy, to survive and to proliferate in extreme environments (well “extreme” from a human point of view of course!)’



a single protein, which was specifically expressed only in the presence of phosphite, and in order to identify and study this new enzyme, they had to develop a new approach that enabled its identification.

Dr Simeonova and her colleagues, Professors Bernhard Schink, Michael Przybiski and Dr Iuliana Susnea, from the University of Konstanz, used a combination of inverted PCR with degenerate primers derived from N-terminal protein sequences and high-resolution peptide mass determination of proteolytic digests from two-dimensional electrophoretic separation. In other words, they adopted a proteomic approach to uncover the genome of *D. phosphitoxidans*. The inverted PCR, which was based on N-terminal sequences and high-resolution peptide mass fingerprinting, provided the identification of a new NAD(P) epimerase/dehydratase by the specific assignment of

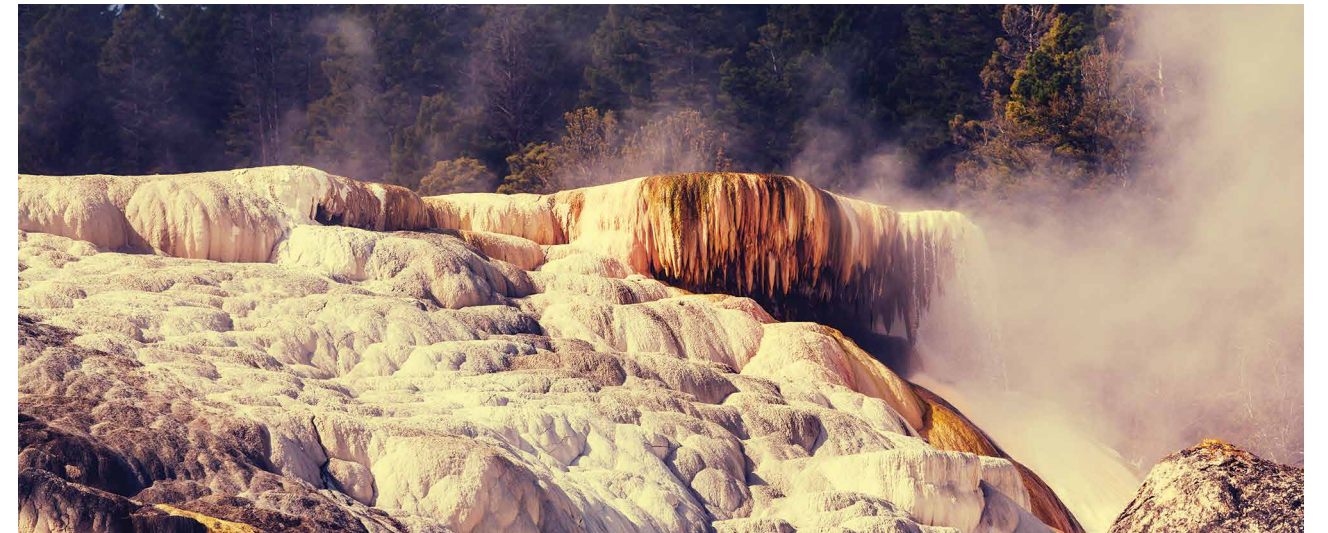
the peptide masses to a single open reading frame (ORF), excluding other possible ORF candidates. They identified the protein using chromatographic separation and sequencing of the internal proteolytic peptides. A metal ion affinity isolation of the tryptic peptides and high-resolution mass spectrometry provided the identification of five phosphorylation sites identified in the domains at locations 23–47 and 91–118 of the protein. In agreement with the phosphorylation sites identified, a direct molecular weight determination of the soluble protein eluted from the two-dimensional gels by mass spectrometry provided a molecular mass of 35,400 Da, which turned out to be consistent with an average degree of three phosphorylations. Ultimately, at the time of this study, *D. phosphitoxidans* was the only bacterium known to oxidise phosphite to phosphate and gain energy from the oxidation process.

Dr Simeonova’s team identified the first protein and its gene involved in this process.

#### The phosphite oxidation gene cluster

As mentioned above, the diversity of the pathways used for assimilatory phosphite oxidation and the fact that *D. phosphitoxidans* is the only known bacterium to use phosphite as an electron source, led Dr Simeonova and her team, in collaboration with Professor W. W. Metcalf at the University at Urbana Illinois, USA, to investigate phosphite uptake and the oxidation gene cluster of this bacterium. They specifically wanted to develop enzymatic assays to measure the phosphite oxidation activity in cell extracts, to identify the genes involved in phosphite uptake and oxidation and to physiologically characterise these genes.

To tackle these aims, Dr Simeonova and



her colleagues constructed a genomic library of *D. phosphitoxidans* using the fosmid vector pJK050. They used PCR to screen for clones harbouring the genes involved in phosphite oxidation using primers that were developed based on the amino acid sequences of phosphite-induced proteins. A sequence analysis of two positive clones identified a putative operon that consisted of seven genes that were predicted to be involved in phosphite oxidation (*ptxED-ptdFCGHI*). The gene encoding the NAD(P) epimerase/dehydratase was named *ptdF*, and the other genes were named appropriately. They cloned four of these genes (*ptxD-ptdFCG*) and heterologously expressed them in *Desulfotignum balticum*, which is the closest relative strain of *D. phosphitoxidans* that does not use phosphite as either an electron donor or as a phosphorus source.

The *ptxD-ptdFCG* gene cluster that was identified was sufficient to confer phosphite uptake and the oxidation ability in the *D. balticum* host strain but it did not permit the use of phosphite as an electron donor for chemolithotrophic growth. The phosphite oxidation activity was measured in the cell extracts of the *D. balticum* transconjugants, suggesting that all of the genes required for phosphite oxidation were cloned. The genes of the phosphite gene cluster were assigned putative functions based on the sequence analysis and enzyme assays.

The gene cluster identified by Dr Simeonova’s group in *D. phosphitoxidans* is the first to show divergence among the phosphite gene clusters of bacteria. At this locus, five new genes were identified. These genes are involved in phosphite oxidation, and this cluster is different from all of the other phosphite oxidation loci so far described, in terms of its structure and the nucleotide sequence of the genes. In addition, it is unique at the amino acid level of the conserved proteins. These data obtained by Dr Simeonova’s team suggest that there might be more variation in the proteins and gene clusters that are involved in sensing, transporting and either the utilisation of phosphite as a single phosphorus source and/or its use as an electron donor in the energy metabolism of bacteria.

#### Obtaining the genome sequence

Alongside the enzymatic assays aiming to discover the energy conservation mechanism and pathway, Dr Simeonova and her team, together with Dr Anja Poehlein and Professor Rolf Daniel from the University of Goettingen, Germany, focused on the cloning and

overexpression of the newly discovered proteins. Their attempts to purify the native proteins involved in the process of anaerobic dissimilatory phosphite oxidation that occurs in the bacteria *D. phosphitoxidans*, led to the sequencing of the genome of the strain. The team revealed that the genome contains 4,998,761 base pairs and 4646 genes of which 3609 were assigned to a function, and 1037 are without a predicted function. Using a metabolic reconstruction, the team revealed that most of the biosynthetic pathways of Gram negative, autotrophic sulfate reducers were present. Additionally, they found and confirmed the ability of the strain to couple phosphite oxidation with dissimilatory nitrate reduction to ammonia, which in itself is a new type of energy metabolism.

Surprisingly, the team only identified two pathways for the uptake, assimilation and utilisation of inorganic and organic phosphonates in the genome. The unique *D. phosphitoxidans* Ptx-Ptd cluster is involved in inorganic phosphite oxidation and an atypical C-P lyase-coding cluster (Phn) is involved in the utilisation of organophosphonates. Ultimately, Dr Simeonova and her colleagues presented the whole genome sequence of the first bacterium able to gain metabolic energy via phosphite oxidation. Dr Simeonova states that ‘one of the most important pieces of information obtained through this work was the ability of *D. phosphitoxidans* to grow by coupling the phosphite oxidation with nitrate reduction under anaerobiosis. This ability was confirmed with physiological experiments.’ In addition, the data also provides the initial information on the composition and architecture of the phosphite-utilising and energy-transducing systems needed to live with phosphite as an unusual electron donor.

#### Future Studies

Based on the fact that *D. phosphitoxidans* is able to couple the phosphite oxidation not only to sulphate, but also to nitrate reduction, Dr Simeonova and her colleagues expect that there must be other bacterial strains that are able to gain energy via dissimilatory phosphite oxidation coupled to other final electron acceptors. Thus, her team is interested in discovering such bacterial species, studying their physiology, biochemistry and energy conservation mechanisms, as well as their spatial distribution in different habitats. Thus, Dr Simeonova’s future work will extend the current knowledge on the biogeochemical cycle of phosphorus and its fluxes in nature.





## Meet the researcher

**Dr Diliانا D. Simeonova**  
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Dr Diliانا D. Simeonova is an assistant professor in the Department of Infectious Microbiology at the Bulgaria Academy of Sciences. She first became fascinated by geomicrobial interactions as a third year student at the University of Mining and Geology, in Bulgaria, where she studied geochemistry and microbiology as separate subjects. This gave her the idea to pursue a research career at the interface between geochemistry and microbiology – or geomicrobiology. Dr Simeonova then went on to receive her PhD from Louis Pasteur University in Strasbourg, France, for a project entitled ‘Arsenic Oxidation of *Cenibacterium arsenoxidans*: Potential application in bioremediation of arsenic contaminated water’. This was followed by a few years of postdoctoral research at the University of Konstanz in Constance, Germany, where she then went on to become the principal investigator on several geomicrobiology projects.

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Dr Anja Poehlein, Georg-August University of Göttingen

Professor Michael Przybyski, University of Konstanz  
Dr Iuliana Susnea, (formerly) University of Konstanz, now a scientist in a private sector company in Rostock, Germany

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DFG

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## UNSEEN WORLDS: MICROSCOPIC LAKE PLANKTON FUEL FOOD CHAINS AND BEFRIEND ALGAE

**Dr Bettina Sonntag** investigates ciliates – microscopic organisms living in freshwater lakes. As a senior scientist at the Research Institute for Limnology in Mondsee, Austria, Dr Sonntag leads a research team exploring how these single-celled protists interact with their environments. Their studies are documenting ciliate diversity and elucidating ciliates’ roles in microbial food webs, including their mutually-beneficial partnership with green algae.

### Ciliate plankton play essential roles in the environment

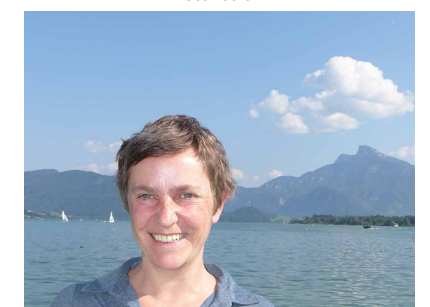
Ciliates are tiny unicellular organisms invisible to the naked eye, present in almost all freshwater environments. ‘Ciliates are named for their cilia – short hair-like appendages used for movement and eating. Ciliates are not animals, plants, or fungi.’ Dr Sonntag explains. ‘Unlike single-celled bacteria and archaea, ciliates’ organelles, the cellular workhouses, are membrane-bound. With a defined nucleus enveloping genetic material, ciliates belong to the protist kingdom.’ Dr Sonntag was drawn to observing these fascinating organisms carry out complex life processes as only a single cell, watching them move, feed, and divide under the microscope. She told us that she is ‘again and again fascinated by the fact that

these organisms consist of only one cell.’

The investigations carried out by Dr Sonntag and her team provide a glimpse into the secrets into the microscopic world of ciliates. Their work is revealing how ciliates and other freshwater protists interact with other organisms. ‘In our studies, we determine the respective food organisms (algal species, bacterial strains, etc.) for a set of planktonic ciliate species, which may then provide an explanatory variable for seasonally changing ciliate diversity patterns.’ Dr Sonntag tells us. Her research seeks to elucidate how ciliates survive amidst the shifting environmental stressors in oligotrophic lakes, harsh ecosystems with low nutrient levels. To carry out these investigations, the team ‘studies ciliates in lakes all over the world including lakes on islands in the Pacific Oceans, soda



Stokesia



Coleps



lakes in Kenya and of course freshwater lakes in Europe.’

Aquatic food webs are a major driving force behind ecosystem functioning. Dr Sonntag and her fellow ecology researchers seek to understand the complex relationships between members of the food chain. She tells us that ‘a major component in aquatic food webs are microscopic organisms, including viruses, bacteria, protists and small metazoans.’ Ciliates and algae are among these microscopic organisms that make up the base of the food web. Despite their size, ciliates are integral to microbial food webs. By preserving environmental balance, ciliates promote the efficient recycling of chemical substances essential to life. For example, by exchanging carbon and oxygen gases with green algae, they help power essential carbon cycles. Ciliates are prey for microbes in higher positions along the food chain, who in turn feed crustaceans and fish.

Understanding ciliates’ contributions to lake ecology must begin at the species level. Dr Sonntag tells us how her work helps advance researchers’ understanding of aquatic food webs: ‘In the past, “protists” were more or less neglected in such studies or lumped into “black boxes” such as “ciliates” or “heterotrophic flagellates”. However, to reveal the complex patterns and interactions in aquatic food webs, the identification of individual species and their ecological traits and specific biotic/abiotic interactions are a prerequisite.’ Dr Sonntag and her colleagues are identifying and investigating specific ciliate species, observing their close relationships with green algae, and advancing the use of state of the art technology to genetically categorise plankton species.

**Happy together: symbiosis between ciliates and green algae promotes survival**

Ciliates and green algae thrive as a pair, and Dr Sonntag’s work has investigated how living together benefits both species. Their relationship is most probably not essential, as both ciliates and the algae can survive independently, but symbiosis protects both against mortality amidst harsh lake environments. As part of this work, the team looked at the ciliate species *Paramecium bursaria* and probed its close relationship with green algae. The pair help each other survive in harsh environments with little available food, and protect each other against starvation in nutrient-poor lakes.

**‘We are building a basic database from morphology up to molecular sequences which can be used by any researcher who investigates lake ecosystems’**



*Urocentrum*

When living in symbiosis with algae, ciliates are mixotrophic organisms, meaning that they continue to engulf food as heterotrophs, but also consume photosynthetic energy from autotrophic algae. With extra carbohydrates from photosynthetic algae, symbiotic ciliates need little external food supply. In turn, ciliates give off respiratory CO<sub>2</sub>, an essential ingredient for algal photosynthesis. With enough light and ciliate-produced CO<sub>2</sub>, algae perform photosynthesis. This cycle produces oxygen by-products that the ciliates can benefit from in turn. As nutrient and sunlight levels are always in flux in oligotrophic lakes as the weather shifts and the seasons change, this exchange between ciliates and green algae increases the survival rates of both species in these harsh habitats.

**Stress responses: algae act as ciliate sunscreen**

Plankton are highly sensitive to light, and life in lakes is often oriented around avoiding sun exposure. In clear lakes, many plankton travel down deeper at around midday when the sun is at its peak. Although sun exposure may damage ciliates, Dr Sonntag did not



*Metopus*

observe such midday migration behaviour in ciliate species from a high mountain lake. Instead, some ciliate species may rely instead on the sunscreens properties of their algae friends.

Both shortwave ultraviolet radiation (UVR) and photosynthetically active radiation (PAR) can cause ciliates harm. UVR passes through cell membranes, causing potentially fatal DNA and protein damage, and ciliates exposed to UVR appear deformed under a microscope. Movement and growth slows, with exposure proving fatal for some ciliates. If a freshwater ciliate population is decimated by excessive sun exposure, aquatic food webs can be thrown into disarray. Furthermore, if a particular ciliate species dies out, the bacterial groups they prey on can rise in number, disrupting ecological balance. The imbalance can have massive implications for the entire habitat.

Dr Sonntag and her colleagues investigated how algae act as a ciliate sunscreen by exposing two groups of a ciliate species – those living in symbiosis with green algae and those living independently from their symbionts – to artificial UVR and

PAR. Symbiotic ciliates fared much better under radiation exposure. Strikingly, the ciliates appeared to huddle together under radiation, forming what Dr Sonntag and her colleagues termed a ‘collective shield’ to protect against sun damage. The green algae living with ciliates migrated to the rear end of cells when light was present. This migration may shield the ciliates’ genetic material. Further investigation is needed to fully understand what triggers these rapid protective reorganisations.

Ciliates and other protists offer a model system for scientists interested in studying the responses to environmental stressors, and future investigations will have much to reveal about how ciliate plankton respond. These microscopic single celled organisms provide highly useful models for studying stress response systems in multi-cellular organisms, including investigations of adaptive stress responses and repair mechanisms.

**Future directions: building a genetic database for ciliates**

The microbial world is incredibly diverse. Lake ecology researchers have historically relied on microscopic observations to identify plankton species and have categorised organisms by structure, size, and movements. This highly variable identification method relies on a trained eye. However, Dr Sonntag and colleagues are pioneering lake ecology by the use of new genetic sequencing technologies. These methods can reveal the DNA code of plankton species in a sample. From these codes, researchers can reliably identify known species, while also discovering unknown species.

Next generation sequencing takes extracts of genetic material and after a series of priming steps, amplifies it to make many copies of DNA so scientists have sufficient amounts of genetic material to analyse. Researchers can then read the sequences of the small DNA fragments generated. Using bioinformatics programs and specialised algorithms, overlapping regions in the small fragments are analysed to re-build complete DNA sequences. Dr Sonntag and her team sought to investigate how well one of the next generation sequencing methods reflected the different types of plankton that were observed via a microscope in a lake sample. Unfortunately, these investigations showed that massive parallel sequencing still has significant shortcomings in the context of protistan research. In a recent study, the team found that nearly two-thirds of morphologically-identified plankton species (observed by looking at the sample under a microscope) failed to be identified by sequencing methods.

There is a missing link in the method: a reliable and thorough database for matching known protist species to their DNA code. Dr Sonntag’s team is leading this effort for ciliates species. ‘We are building a basic database from morphology up to molecular sequences which can be used by any researcher who investigates lake ecosystems,’ Dr Sonntag explains. A complete genetic database will allow for rapid assessment of the species present in a freshwater sample. Dr Sonntag tells us that researchers expect there are around 150 ciliate species in the plankton of one lake, and ‘planktonic ciliate species have already been described from morphology,’ however ‘knowledge on their ecology or molecular sequences are still awaiting to be discovered.’ Observing plankton population levels across seasons and in extreme environments would also become easier with a complete genetic database. DNA code matching also will distinguish between species appearing morphologically similar under a microscope. This effort can help researchers identify new species, unearthing more plankton diversity.

**‘In our studies, we determine the respective food organisms for a set of planktonic ciliate species, which may then provide an explanatory variable for seasonally changing ciliate diversity patterns’**



*Teuthophrys*

Dr Sonntag and colleagues also hope that DNA sequencing can lead to better understanding of rare ‘seed bank’ plankton. Plankton are highly sensitive to environmental conditions that alter nutrient availability and levels of light. Microscopic lifeforms respond quickly to such changes, reproducing or becoming dormant. The special ‘seed bank’ species spend most of their time in a dormant state, activating and blooming under certain environmental conditions only. A lake’s ciliate diversity is constantly in flux, and these ‘seed bank’ species provide an important buffer against environmental changes, allowing plankton to replenish population levels after environmental shocks. These rare species are estimated to make up less than 0.1% of the plankton population, making microscopic observation extremely difficult. Next generation sequence methods may hold the key to unlocking the secrets of the ‘seed bank’ plankton, giving us further insight into how these incredible protists weather the cycles of life in harsh freshwater lakes.





# Meet the researcher

**Dr Bettina Sonntag**  
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Dr Bettina Sonntag was awarded her PhD from the Leopold-Franzens-University Innsbruck (LFUI) in Austria in 2000. Since then, she worked for several years as a postdoctoral researcher at the Institute of Ecology in Innsbruck and at the Research Institute for Limnology, Mondsee, at the same university. As a senior scientist, she now leads her own research team at LFUI that investigates ciliate species in lakes, which play integral roles in microbial food chains. Her research group has made large strides in understanding freshwater planktonic ciliates and in low-nutrient lake ecosystems. Over the course of her career, Dr Sonntag has been presented with many awards, including the Kanadapreis from the Canadian Studies Center of the LFUI for published work on ciliate-algae symbiosis.

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## FUNDING

Austrian Science Fund FWF: projects P21013-B03, I2238-B25 and P28333-B25 (PI B. Sonntag)  
Leopold-Franzens-University Innsbruck  
German Research Foundation  
DFG: project STO414/13-1 (PI T. Stoeck)  
Swiss National Science Foundation SNF: D-A-CH project 310030E-160603/1 (PI T. Posch)

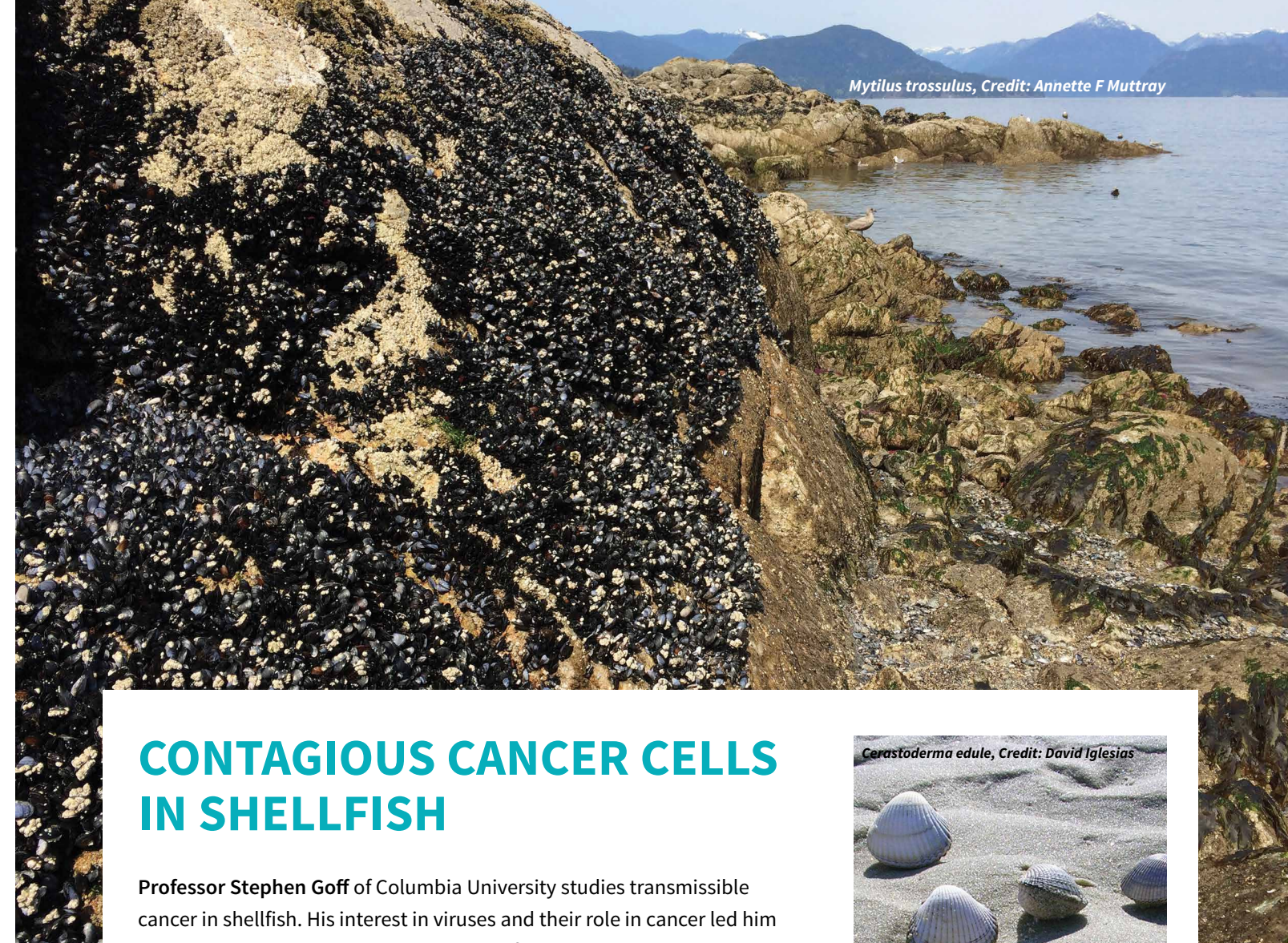
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*Mytilus trossulus*, Credit: Annette F Muttray

## CONTAGIOUS CANCER CELLS IN SHELLFISH

**Professor Stephen Goff** of Columbia University studies transmissible cancer in shellfish. His interest in viruses and their role in cancer led him to initially research a potential viral cause for leukaemia that is present in shellfish. The cancer did not have a viral origin, but to his surprise, it emerged that the disease was directly transmissible from shellfish to shellfish through contagious cancer cells.

### Cancer – not traditionally transmissible

Cancer is a disease of uncontrolled cell proliferation, migration and resilience that is typically restricted to the organism in which it originated. Tumours arise in various tissues as a consequence of genetic mutations. Such mutations sometimes occur spontaneously and sometimes are induced by carcinogenic toxins or viral infections. When these mutations confer the ability to proliferate in an uncontrolled fashion, evade detection by the immune system, invade other tissues, and prevent cell suicide programs that would otherwise kill malfunctioning cells, the result is a malignant, metastatic cancer. While cancer cells often invade other tissues within the same individual, typically they do not spread from individual to individual. As bad as cancer is, it doesn't quite induce the same unease as contagious

diseases such as those caused by Ebola or HIV. It is perceived as a personal disease. People speak about beating their cancer as a personal struggle, whereas contagious diseases are 'fought' on a societal level – just look at the huge mobilisation to contain the latest Ebola outbreak, or the hysteria and stigma that surrounded the HIV/AIDS epidemic of the 1980s. Consequently, for some, the concept of contagious cancer may add an unwelcome dimension to this already feared disease. Such transmissible cancer is the subject of some of the most recent research of Professor Stephen Goff of Columbia University. No contagious cancers are known to affect humans, but examples have been observed in a handful of other species: Tasmanian Devils, dogs and certain shellfish, including clams, mussels and cockles. In Tasmanian Devils, transmissible cancer presents as facial tumours, which are



*Cerastoderma edule*, Credit: David Iglesias

spread when the animals fight each other and inflict and receive facial bites. In dogs, the tumours are spread by mating, making for a particularly nasty venereal disease. Goff has been instrumental in characterising the basis for transmissible cancer in shellfish. However, his research was not originally focused on transmissible cancers, but rather towards tumour viruses and the mechanisms by which they cause cancer.

### How Professor Goff came to study transmissible cancer

Goff began his research career developing methods for construction of recombinant DNAs. His graduate work centred on genetic studies of the Simian Virus 40 (SV40), a DNA tumour virus, and its use as a vector for gene transfer into mammalian cells. In his postdoctoral work in the laboratory of Dr David Baltimore, he became fascinated with retroviruses, of which HIV-1 is an example, and what they can tell us about basic cellular biology. Viruses operate by 'hijacking' cellular



## ‘I think work on retroviruses will continue to reveal new aspects of biology and genome evolution’



machinery in an infected cell to force the cell to produce more viral particles. These new viral particles are released and spread to infect more cells. However, retroviruses do not just commandeer cellular machinery – they actually insert and integrate their genetic material into the DNA of the infected cells. Sometimes this insertion can disrupt the DNA in ways that cause cancer. This is called insertional mutagenesis. These viruses can also recombine with cellular genes and incorporate them into their own genomes, generating transducing viruses that transfer the genes into infected cells. Goff tells Scientia about his early motivations for viral research and its applicability to our understanding of molecular biology and cancer: ‘I’ve been interested in viruses (phage, DNA viruses, and retroviruses) throughout my career. I consider them wonderfully efficient at exploiting cell machinery for their needs, and therefore handy in revealing the most interesting pieces of that machinery. I was motivated to focus on retroviruses by their amazing life cycle – their ability to make DNA copies

of an RNA genome and insert that DNA permanently into the host genome. The study of these viruses has revealed many aspects of cellular molecular biology, and has had a huge role in developing our current understanding of cancer.’

### Cancer transmission in shellfish

Goff was asked by a marine biologist to help investigate if a cancer found in the circulatory system of molluscs, called disseminated neoplasia, had its basis in a viral infection. The cancer is predominantly lethal to bivalves, which include clams, cockles and mussels. Goff told us about how he became involved in studying shellfish cancer: ‘I was recently led into studies of a leukaemia-like disease in molluscs, disseminated neoplasia, by Carol Reinisch, a marine biologist at Woods Hole Oceanographic Institute. She solicited our help in exploring whether a retrovirus might be involved in this disease.’ The team began examining cancer samples from shellfish to determine if the cancer had a viral origin. What they discovered surprised

them: ‘Our examination of the tumour cells found no viruses, but instead revealed high levels of expression of an endogenous retroelement (which we named Steamer). The element had undergone a dramatic copy number expansion in the tumour cells, and examining the sites of integration of the new copies gave us a big surprise: many integration sites in tumour DNA isolates from disparate locations were identical, suggesting that the tumours were related. Genotyping the tumour DNAs confirmed that all the tumours were derived from a single clone – and that this tumour genotype did not match that of the host animals.’ Given that the tumours did not match the genetic makeup of the molluscs, and tumours from different molluscs were genetically similar, the team were left with only one conclusion: ‘The tumours did not arise in the usual way, by mutations in somatic cells of the host, but had spread between animals as a contagious disease. Since then we have found similar tumours spreading in other molluscs.’

## ‘The tumours did not arise in the usual way, by mutations in somatic cells of the host, but had spread between animals as a contagious disease’



### Interspecies transmission

The team have also discovered an even stranger case of a transmissible cancer – one that can cross the species barrier. The team analysed the cancer found in a type of clam called the golden carpet shell. In analysing the genetic makeup of the tumours they found that the tumours did not arise from a different individual of the host species – but rather they derived from an individual of a distinct species called the pullet shell clam. To make things even stranger, this cancer is not commonly found in the present pullet shell clam population. The team think that the cancer originated in the pullet shell and spread into the golden carpet clam, and that the original species may have then developed resistance to the tumours over time and so is not afflicted by the disease today.

Recorded instances of cancer transmission are relatively rare – the shellfish and mammalian examples discussed in this article comprise the extent of known species for which cancer transmission has been recorded. However, Goff believes that it may be more common than we think and that new examples are just waiting to be discovered.

### Transmission of cancers – how do they spread?

So given that there is clear evidence of transmissible tumours between certain shellfish, how do the tumours get from one individual to another? This is a question that the team want to answer. In the

mammalian examples of transmissible tumours, transmission can be clearly linked to physical contact, either through fighting or mating (which, if we’re honest, are two of the all-time favourite activities of most mammals). However, in the marine environment, where there is limited physical contact between shellfish it appears that the water itself may act as a conduit for contagious cancer cells to spread and infect new hosts. Infected molluscs might release infectious cells in their faeces, which could easily be picked up by other molluscs, thereby ‘seeding’ them with new tumours. Molluscs feed by filtering food from seawater and so might be particularly susceptible to ingesting infectious cells in the water. Molluscs are not thought to have a very sophisticated immune system, and may be incapable of rejecting introduced tumour cells with a genetically different makeup. Humans possess a much more sophisticated immune system, which can identify and reject a variety of foreign tissues and pathogens, so it is perhaps unlikely that human cancers could become transmissible. However, cancer transmission has been possible in some mammals, such as the dogs and Tasmanian Devils mentioned previously, in special settings. They may be the victims of an unfortunate and highly unlikely set of circumstances, whereby a tumour with just the right biological characteristics to avoid inter-individual immune rejection developed and had the opportunity to spread through inter-individual physical contact. In human patients who are severely immunocompromised, or between patients who are genetically very similar, such as identical twins, there is perhaps more scope for cancer transmission, although the risk is likely to still be very low, since a superficial tumour, physical contact and perhaps broken skin on the recipient tissue would likely all be required for successful transmission.

### Future work

Goff talked to Scientia about his hopes for future research on viruses and their impact on cancer: ‘I think work on retroviruses will continue to reveal new aspects of biology and genome evolution. In my lab we are looking to identify and characterise new host gene products that impact on retrovirus replication: factors that either inhibit their spread, or are exploited by the viruses to promote their spread. These are telling us about the host responses to virus infection, and the way we hosts are adapting to (and incorporating) virus genomes in our species. They are also specifically telling us much about cancer, because of their ability to directly induce cancers, and because of their highlighting the conserved pathways that lead to non-viral cancers.’

He also spoke about his plans for further research into transmissible cancer: ‘Broadly, my lab will continue to study the replication of retroviruses, and search for new aspects of the virus-host interaction. With respect to the contagious cancers in molluscs, we are interested in identifying the mutations that led to the initial oncogenic clone; and those that are involved in its ability to spread and colonise a new host individual. We are also interested in the basis of restriction of most of the tumours to spread within the species of origin, and the explanation for the rare cases of interspecies transmission that we have detected. We hope these efforts may tell us about the primitive immune system of these animals that we suppose may be actively limiting spread of the contagious clones.’





## Meet the researcher

**Professor Stephen Goff**  
College of Physicians and Surgeons  
Columbia University  
New York  
USA

Professor Stephen Goff obtained his PhD from Stanford University in 1978, following which he pursued postdoctoral research at the Massachusetts Institute of Technology. He is currently Higgins Professor in the College of Physicians and Surgeons, Columbia University and an Investigator of the Howard Hughes Medical Institute. He has authored or co-authored in excess of 300 publications, is a member of the National Academy of Science, the National Academy of Medicine, and the American Academy of Arts and Sciences, and a fellow of the American Association for the Advancement of Science. He studies the life cycle of retroviruses, and the mechanisms by which they cause cancer. His most recent work has involved the study of transmissible cancers in shellfish.

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### KEY COLLABORATORS

Goff is presently working on intracellular virus trafficking together with Gregg Gundersen and Richard Vallee, colleagues at Columbia University, and Mojgan Naghavi and Derek Walsh at Northwestern University in Chicago. Goff maintains active collaborations with three of his former postdoctoral fellows now heading active laboratories of their own in China: Guangxia Gao at the Institute of Biophysics at the Chinese Academy of Sciences in Beijing, Baojie Li at the Bio-X Institutes and Shanghai Jiao Tong University in Shanghai, and Yong Cang at Zhejiang University in Hangzhou. His recent work on transmissible cancers in molluscs has been a collaboration with Carol

Reinisch, formerly at the Marine Biological Laboratory at Woods Hole, Massachusetts and James Sherry at Environment Canada, Water Science & Technology Directorate in Burlington, Ontario.

### FUNDING

Goff is supported by the Howard Hughes Medical Institute, and by grants from the National Cancer Institutes and National Institute of General Medical Sciences of the National Institutes of Health.

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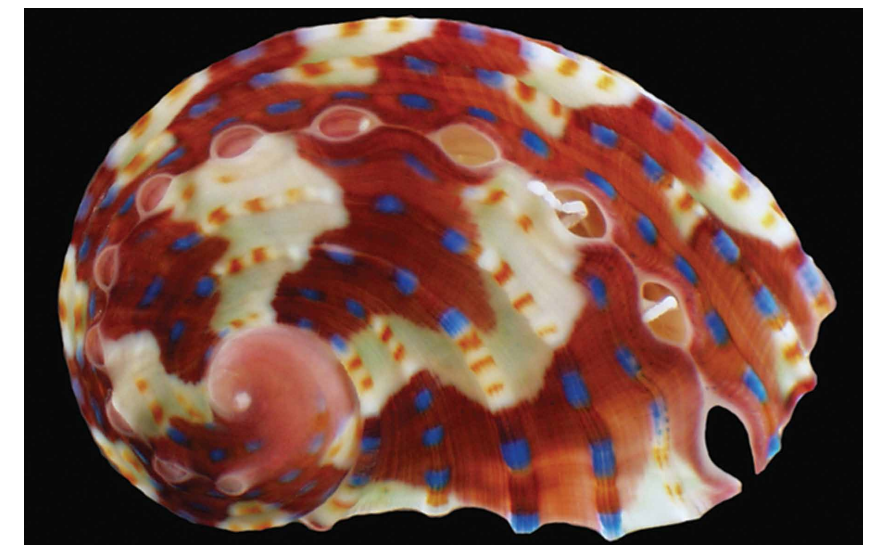
## HOW ANIMALS FABRICATE BIOMINERALS

**Professor Daniel J. Jackson** and his team at the University of Göttingen use snails and sponges as models to study how animals make biominerals, and to gain insight into how this ability first evolved. The cellular and molecular mechanisms that allow an animal to make a shell, a spicule, a tooth or a spine are incredibly complex and poorly understood in all but a few animals. However, the evolution of this ability, more than 540 million years ago, allowed early animals to precipitate calcium and other minerals in highly controlled and useful ways. This is likely to have provided key support for the rapid and widespread diversification of animal life in an unprecedented evolutionary event known as the Cambrian Explosion.

### Biomimetalisation

If you trace the phylogenetic tree of life back far enough, you will find that animals as complex as humans share a common ancestor with animals such as earthworms, corals, insects and all other animal life forms. Along this journey back in time a multitude of evolutionary events took place, some of which fundamentally influenced the diversity of animal morphologies that we see today. One of these key events was an innovation – the ability to ‘biomineralise’, or the ability of a living organism to synthesise mineralised body parts by combining organically derived molecules (for example proteins) with minerals available from the environment. Biomimetalisation ranges from a sponge’s capacity to precipitate silica and a snail’s ability to make a calcified shell, to the complex organisation of the human skeleton. Understanding which genes and cellular processes regulate these processes in diverse animals, and whether they share any commonalities is one research theme that occupies Professor Jackson’s group.

Biomimetalisation occurs in representatives of all animal groups, and is responsible not only for the fabrication of endo- and exo-skeletons and the structurally supportive roles they play, but also structures that play sensory, predatory and protective roles too. ‘At some point in time, more than 543 million years ago, a variety of environmental,



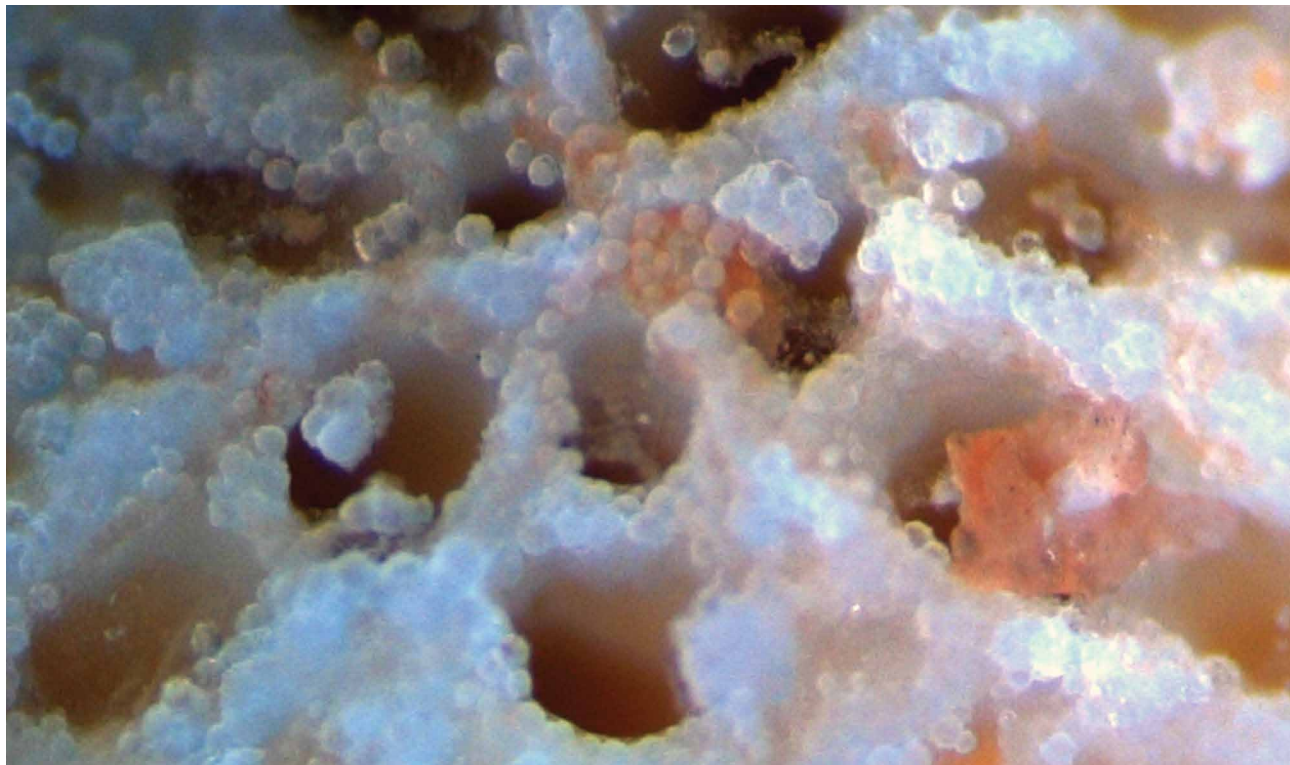
biological and ecological factors acted together to pressure our soft-bodied animal ancestors to evolve the ability to make useful biominerals,’ Professor Jackson tells us. Then, very suddenly and synchronously (geologically speaking), there was a very rapid and sustained increase in the diversity of animal life forms. This particular period in the history of animal evolution has come to be known as the ‘Cambrian Explosion’. ‘Because of the close association between the evolutionary appearance of an ability to biomineralise, and the rapid and synchronous increase in the diversity of animal forms during the Cambrian, an understanding of the animal origins of biomineralisation would provide insight

into how the diversity of animal life that we observe today came to be,’ says Professor Jackson.

Advances in scientific technologies, such as whole genome sequencing, have better allowed scientists to study cellular and biological processes at a genetic level, and to make comparisons between animals in order to identify where changes and deep conservation have occurred. By first identifying which genes are directly involved in making a shell, a spicule or a spine, Professor Jackson and his colleagues hope to develop gene-specific in vivo functional assays that will allow them to further characterise the differences in



**‘A general theme of our work is to understand to what degree animals use the same genes and cellular processes to build their biominerals. This in turn should help us to understand some of the molecular details that accompanied the Cambrian Explosion and the generation of diverse complex animal life’**



biomineralisation programs operated by different species. This will allow them to understand, for example, how a mollusc makes its shell and how this ability first evolved.

**Accidental discoveries in the genetics of shell formation**

Professor Jackson stumbled into the field of biomineralisation during the final phases of his PhD. He was initially seeking to identify some of the genes that coordinate the transformation of larval marine invertebrates into benthic adults. In his search, he identified two novel genes expressed in larvae preparing to metamorphose into the next phase of development. This is a critical and vulnerable stage during the development of many invertebrate species, and therefore has significant implications for global food production, particularly in countries where the primary protein source lies within the aquaculture industry. The specific model organism that Professor Jackson discovered these genes in was a commonly aquacultured mollusc, the

abalone, and the genes of interest were highly expressed within the tissue that forms the shell. ‘The expression patterns of these two genes in the abalone larva were so beautiful, and so unique that they raised many interesting questions that weren’t on our radar at the time. I was immediately hooked and had to know more about what these shell-forming genes were doing, and what their origins were,’ he recalls.

**Sponges and skeletal synthesis**

One question that has long occupied the scientific community about the Cambrian Explosion is ‘why did it occur?’ Many hypotheses exist, including but not limited to the generation of novel genes and microRNAs, increasing ecological interactions between various previously isolated species, and changes in ocean water chemistry. However, none of these hypotheses answer the question of how and why so many individual organisms developed the ability to fabricate such a variety of mineralised structures. ‘Sponges are a very useful group of animals with which to address these kinds

of questions,’ Professor Jackson explains. ‘If we can identify a cellular pathway, gene or process that is used by a sponge to biomineralise, and if a mollusc, or any other animal, also uses that same feature to build its skeleton, there’s a good chance that the last common ancestor of those two animals (which must have lived more than 543 million years ago) provided that feature to all of its descendants.’ Using this comparative approach, Professor Jackson and his group recently used a sponge to demonstrate that the autophagy (‘self-eating’) pathway is utilised by at least one species of sponge to build its skeleton. Autophagy is not a sponge-specific or novel concept. It has proven involvement in both pathological and non-pathological processes, such as infection, cancer, arthritis, starvation and programmed cell death, and a Nobel Prize was awarded this year for its discovery. However, the connection between autophagy and biomineralisation is new. This idea could also be connected to the fact that early on in the Cambrian Explosion, seawater calcium concentrations rose to levels that would most likely have been dangerous to the

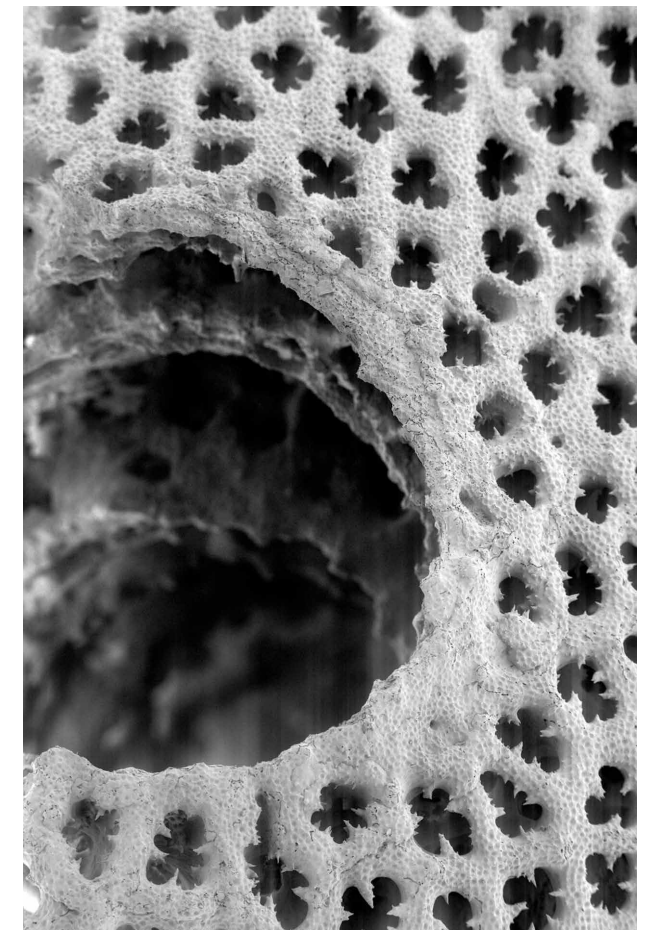
cells of our distant animal ancestors. Professor Jackson theorises that some organisms may have developed biomineralisation strategies as a means to rid themselves of these toxic levels of calcium by employing the evolutionarily ancient autophagy pathway.

Professor Jackson and his group also used the same sponge to identify a ‘calcification’ gene with a deep evolutionary history. The enzyme carbonic anhydrase, allows cells to combine carbon dioxide and water to produce bicarbonate and a proton, and is one of the most efficient enzymes known. This reaction, found ubiquitously in cellular metabolism and other processes, helps regulate pH, process metabolic wastes, fix carbon, and transports ions across membranes in all animals. Sponges are one of the earliest branching animals to secrete a calcium carbonate skeleton, and Professor Jackson has shown that sponges inherited a carbonic anhydrase gene from the last common ancestor of all animals, and that one version of this gene is still to this day employed by the sponge to build its skeleton. In another project, he demonstrated that another biomineralisation protein used by the same sponge was long ago horizontally transferred from a bacterium into the genome of the sponge. This work highlights the ancient and intimate association that exists between sponges and bacteria. It also draws a link back to the autophagy work, as it is this pathway that the sponge employs to degrade endobiotic bacteria. The degraded remains of these bacteria are then used as a template for biomineralisation by the host sponge.

‘While sponges are extremely informative because of their phylogenetic position on the animal tree of life, they are not the easiest model to work with on a day-to-day basis,’ says Professor Jackson. ‘They can be tricky to keep in an artificial environment (such as in the middle of Germany), and they don’t reproduce predictably, or on a scale that would support most of our research needs. For these practical reasons we turn to molluscs.’ Being the second most speciose phylum on the planet, the diversity of molluscan shells available for study is vast. They can also be used for many other fascinating research questions.

**What causes asymmetry?**

What causes most animals to develop an asymmetry about the left and right sides? While we like to think of ourselves as symmetrical about our mid-line, the reality is there are many differences between our right and left sides. For example, we carry our heart, spleen and pancreas on the left, while our brains display complex neurological asymmetries that we are only beginning to understand. What are the genes that control the process of establishing left-right asymmetry early in life, and are they the same genes used by all animals? A focused international collaboration involving Professor Jackson and led by Professor Angus Davison (The University of Nottingham, U.K.) identified a single mutation that causes snails to coil their shell in the opposite direction to that normally observed. Formin, a highly conserved cytoskeletal protein, may be largely responsible for determining left-right patterning in both molluscs and vertebrates, including humans. Using the pond snail as a model, the team discovered that a disabling mutation in the formin protein is associated with ‘symmetry breaking’ events in embryos of the pond snail. By administering a specific ‘anti-formin’ treatment to normal snail embryos, chirality was reversed. The same treatment randomised chirality in developing frogs. This study highlights the value in using nonconventional model organisms and supports the notion that asymmetry is a highly conserved, ancient property shared by all complex animals.



**Evo-devo and its relevance to an integrated understanding of biomineralisation**

Evo-devo, or evolutionary developmental biology, interprets developmental processes within an evolutionary framework, and Professor Jackson emphasises that conducting his studies through this lens is critical to the advancement of his research within the field of biomineralisation. Because biomineralisation begins very early in life for most animals, the developmental programs that coordinate, regulate and initiate the deposition of mineralized structures hold a great deal of information for the evolutionary biologist. ‘While we know those first genes we discovered in abalone larvae are somehow involved in biomineralisation, we unfortunately still don’t know exactly what they’re doing,’ Professor Jackson explains. He and his colleagues intend to continue in the same direction with the hopes of harnessing the rapidly evolving molecular technologies available to the scientific community. ‘Exciting new technologies are now becoming available that should allow us to start revealing the functions of these novel genes in vivo,’ Professor Jackson tells us. The development and availability of new genome editing methods should allow researchers working with nonconventional model organisms to begin to interrogate and interpret the wealth of sequence data they have had access to for some years now. By establishing these new technologies in our model organisms, Professor Jackson’s team will continue to study and compare the complex genetic interactions and pathways responsible for the production of molluscan shells, with the aim of ultimately shedding some light on the molecular events that supported the evolution of all animal life.





# Meet the researcher

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Daniel J. Jackson achieved his PhD in 2004 from the University of Queensland, Australia, for a thesis focused on developing applied and molecular approaches to improve the cultivation of the tropical abalone. From this work he became fascinated by the genetic mechanisms snails and oysters use to build their shells, and pursued postdoctoral research under this theme at the University of Queensland and then the University of Göttingen, Germany, prior to becoming a Junior and then Full Professor in the Department of Geobiology, University of Göttingen. He now teaches numerous graduate and undergraduate courses, has supervised over 20 students and postdoctoral researchers, and collaborates with evolutionary biologists worldwide to unveil the intricacies of how invertebrates biomineralise.

## CONTACT

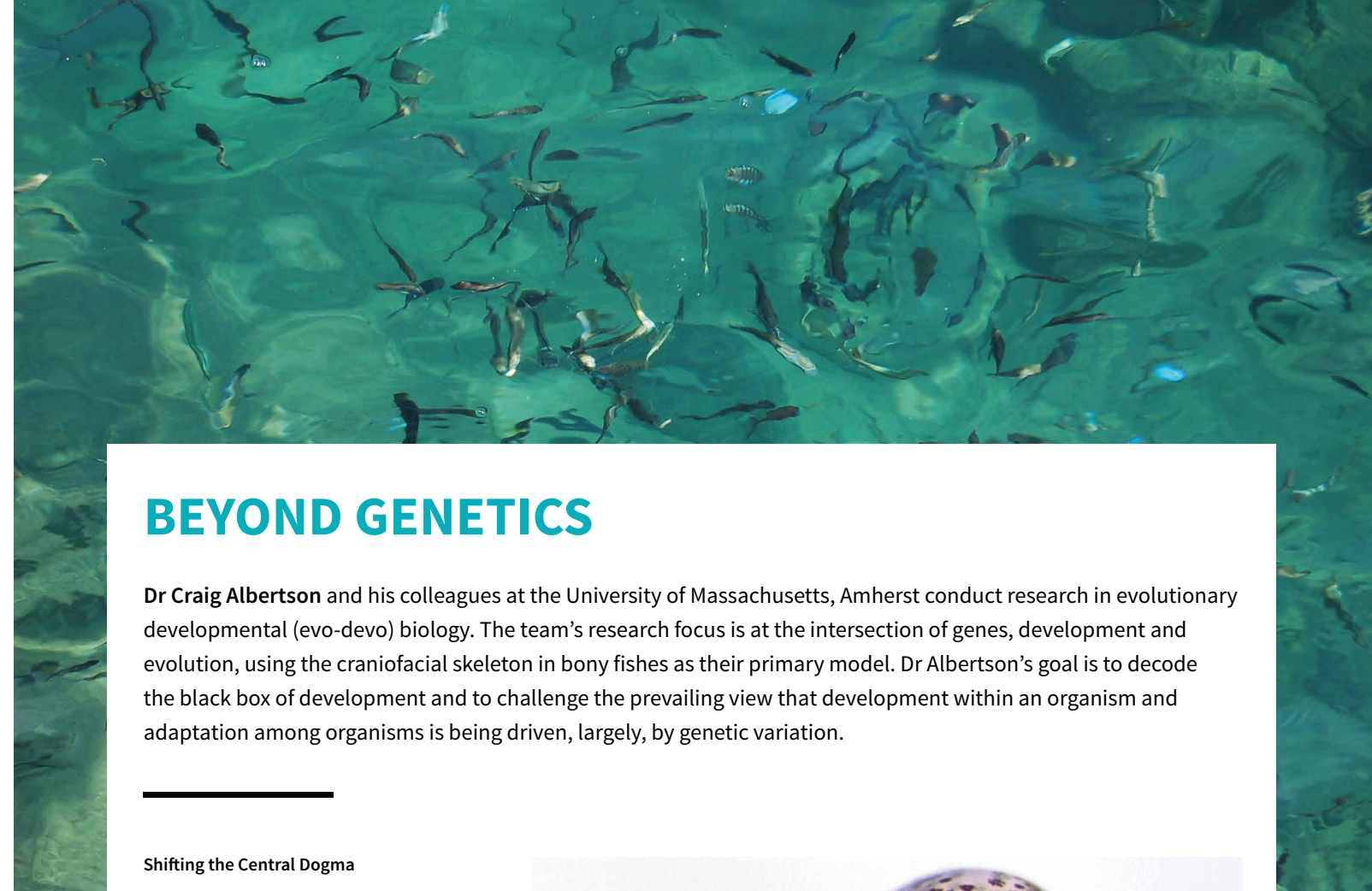
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## FUNDING

Deutsche Forschungsgemeinschaft (DFG)  
VolkswagenStiftung

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## BEYOND GENETICS

**Dr Craig Albertson** and his colleagues at the University of Massachusetts, Amherst conduct research in evolutionary developmental (evo-devo) biology. The team's research focus is at the intersection of genes, development and evolution, using the craniofacial skeleton in bony fishes as their primary model. Dr Albertson's goal is to decode the black box of development and to challenge the prevailing view that development within an organism and adaptation among organisms is being driven, largely, by genetic variation.

### Shifting the Central Dogma

The classic Central Dogma of biology provides a pathway for how genetic information flows from DNA to yield a phenotype. This flow follows the transcription of DNA into RNA, which is subsequently translated into protein. Thus, as plainly stated, the Central Dogma recognises a gene-centric view of a biological system. This means that, based on the Central Dogma, all of the information necessary to define the state of an organism is contained in the sequence of its genome. This is actually a pretty fair assessment of molecular biology when one is describing simple single-cellular organisms, such as bacteria. However, this gene-centric view does not hold up when studying complex multicellular organisms. It is well known for example that monozygotic twins, with identical genomes, can act, behave and even look quite different. Clonal lines of plants grown under drought or wet conditions will display very different and stable phenotypes. Butterflies that metamorphose in spring will have more dark pigment on their wings compared to the same species that metamorphose in the summer. These observations suggest that there is further information that is responsible for the production and maintenance of phenotypes.



Given that the Central Dogma does not provide a complete explanation for biological processes in various fields of biology, many disciplines are shifting this paradigm to better describe biology. One such discipline is evo-devo – a field of biology that combines evolutionary biology and developmental biology. Many researchers in this field believe that the basic Central Dogma cannot define complex traits; for example, if gene expression was simply occurring as the Central Dogma describes, then scientists

could reconstruct a human face from a DNA sample. This would mean that DNA from a crime scene could be used to generate a facial image of a suspect, the remains of prehistoric people could be used to reconstruct their faces or the face of a baby could be predicted before birth using DNA from the amniocentesis. This is an exciting idea, but in reality it's actually quite far-fetched. There are several reasons that DNA alone cannot predict facial shape.



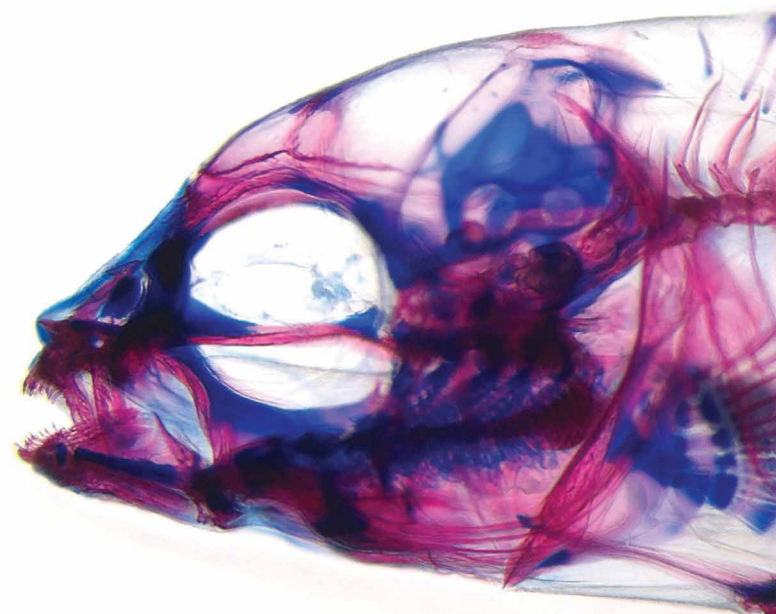
First, a large number of genes have been identified for their involvement in the development of the head, but there is no direct evidence that these genes play a role in the normal variation of the face. As Dr Albertson explains: 'For instance, if you mutate a gene in a mouse, and you end up with a mouse with no head, you may deduce that that gene is necessary for building a head. But it tells you nothing about how the head is shaped over development. Why do some people have longer mandibles while others have shorter mandibles, for example? This represents a major gap in our knowledge, and likely requires the combined action of both genes that the environment.'

It's likely that many genes that are not known for their role in early craniofacial development contribute to normal variation in the face. This is because the genes that contribute to development are most active in the first days or weeks of embryological development (depending on whether you are a fish or a human), whereas variations can arise during both early (e.g., patterning) and later (e.g., growth) processes. Thus, variations in facial shape may come from unanticipated genetic sources.

Another thought provoking notion is that genes play a very minor role in the development of complex morphologies. For example, while it is clear from decades of research that genetic variation causes dissimilarities at the phenotypic level, for complex morphologies such as the facial skeleton genetic variation can only explain a small percentage of the total phenotypic variation, typically much less than 50%. This means that the majority of variation in such phenotypes arises from different sources.

It is becoming increasingly evident that explanations in biology, and specifically evo-devo, cannot purely rely on the Central Dogma to explain the complex biological processes that permit the development of complex traits like the skeletal system. These are the types of questions that Dr Albertson has been interested in from a young age, when he worked as an assistant to an oral and maxillofacial surgeon. It was during this time that he first gained an appreciation for the skull as an organ of unique complexity: 'I asked myself, how did that happen? How does the skull arise over development? How did the vertebrate skull evolve from an invertebrate "head"? How does variation in skull shape arise within and between populations?'

## 'As it turns out, genes cannot tell the entire story. Rather, we want to know how genetic and epigenetic processes combine to tell the story of life, of development and evolution.'



In driving toward answers to these questions, Dr Albertson and his team focus their studies on the genetic and epigenetic (i.e., other than genes) processes that contribute to craniofacial development and evolution. Some current research directions are highlighted below.

### Variation in a single locus can alter multiple aspects of a dynamic system

Traditionally, the focus of evolutionary developmental biology was to link genes with straightforward shifts in morphology, but in order for a species to adapt to new environments, a divergence in resource use must also occur. This divergence often involves variation in multiple components of complex functional systems. Based on this idea, Dr Albertson and his colleagues predicted that, for evo-devo research, the elucidation of broad evolutionary processes should investigate more dynamic traits within this framework.

Dr Albertson's work focuses on the diversification of craniofacial morphology and during vertebrate evolution. The adaptive variation of craniofacial structure occurs in response to different food sources and habitats, which in turn plays a role in niche partitioning and speciation. Actually, a majority of the morphological and functional divergence between vertebrates occurs in the craniofacial region. Thus, it's no surprise that numerous studies have examined the patterns of craniofacial divergence in a wide variety of animals. Of the animals studied, East African cichlids are considered an excellent system to study the genetic and developmental mechanisms that promote craniofacial divergence, because they exhibit a large degree of variation in craniofacial morphology.

To study variations in the craniofacial skeleton, Dr Albertson and his team combined traditional quantitative trait locus mapping, population genetics and experimental embryology to understand how differences in gene expression during larval

development in African cichlids contributes to adaptive morphological variation in adults. In one study, Dr Albertson's team showed that a gene in the Hedgehog (Hh) signalling pathway mediates widespread variation in bones that affects the kinematics of lower jaw depression in cichlids. This seemingly simple action is controlled by a complex arrangement of bones and tendons, and the efficiency of this system has major implications for how well an animal can feed. This finding contributes to the one of the key questions in evolutionary studies, which asks how genetic variation translates into ecomorphological adaptation and ultimately, fitness. Dr Albertson's study presents experimental data that variation in a single gene affects various aspects of a dynamic mechanical system. Ultimately, this work links adaptive variations at genetic, developmental and functional levels.

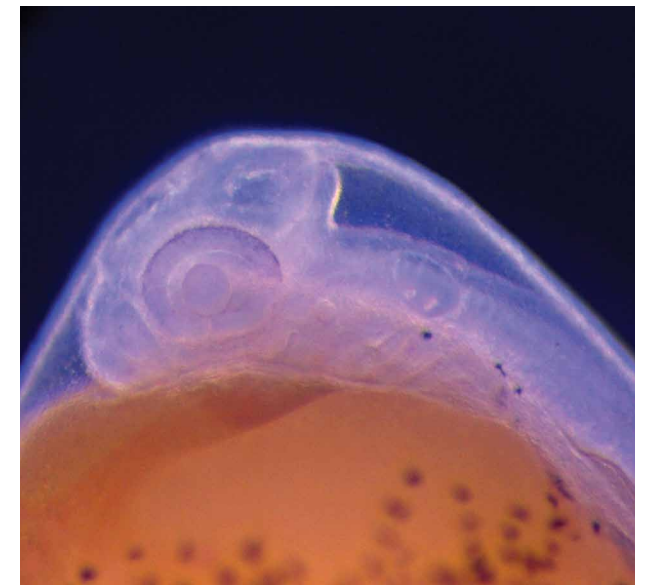
### Epigenetics – a divergence from the Central Dogma

Epigenetics is the study of cellular and physiological phenotypic trait variations that are caused by external or environmental factors that turn genes on and off. While the study above highlights the genetic roles for adaptive variation in the jaw, these genetic effects only contribute to a relatively small percentage of the phenotypic variation that is observed. Cichlids rapidly evolve and display a large spectrum of variation in their craniofacial skeletons, and Dr Albertson is interested in how these changes occur at the genetic level. However, his team also found that there is an epigenetic origin for the adaptive variation that occurs in the cichlid jaw.

The team revealed that cichlid larvae begin gaping their mouths right after the cartilaginous lower jaw forms, which is just prior to the commencement of bone development. They showed further that the frequency of gaping varies between species in a manner that predicts variations in bone deposition. Moreover, when the gaping is disrupted in the fast gaping species, the jaw forms in a manner that is similar to the slow gaping species. In contrast, when the gaping is forced to occur at a higher frequency, the jaw of the slow gaping species develops to resemble that of the fast gaping species. Finally, Dr Albertson and his colleagues showed that these epigenetic changes also occur through the Hh signalling pathway. Thus, both the genetic and epigenetic path to jaw shape variation appears to leverage the same pathway. Taken together, these results emphasise the complexity of how craniofacial shape takes place and propose a novel experimental framework for investigating sources of phenotypic variation beyond those determined by changes in gene sequences.

### Environmental influences on gene deployment during trait development

Phenotypic plasticity refers to the ability of organisms to alter their phenotype in response to changes in the environment. Recently, phenotypic plasticity has been a hot topic in discussions of evolutionary potential, but a complete understanding of the genetic basis of plasticity is lacking. Dr Albertson and his team investigated the genetic basis of phenotypic plasticity in cichlid fishes. They crossed two divergent species to generate a genetic mapping population. These hybrid families, at early juvenile stages, were split and raised in alternate foraging environments that mimicked benthic/scraping or limnetic/sucking modes of feeding. The different environments produced variations in morphology that were largely comparable to the major axis of divergence among the cichlids, which supported the flexible stem theory of adaptive radiation – a theory proposing



## 'While we know a lot about how the head develops, we know much less about how the complex geometry of the skull is determined'

that patterns of plasticity in a population will shape future patterns of morphological evolution. In addition, the study revealed that the genetic architecture of nearly every morphological trait examined was highly sensitive to the environment. In other words, different genes underlie shape variation in different environments. These results have major implications for research aimed at identifying the genes that underlie species divergence. They suggest that results could be misleading if the experiments are not performed in the 'correct' environment, which is to say the environment in which evolutionary divergence occurred.

Natural selection acts upon phenotypic variation. This notion has not changed since the time of Darwin. Recent work in Dr Albertson's lab highlights the importance of genes in generating variation, however it also underscores the immense significance of the environment. As the field moves forward, it will be necessary to incorporate both variables (genes and environment) into a common framework in order to gain a comprehensive and mechanistic understanding of how species evolve.





# Meet the researcher

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Dr Craig Albertson is an Associate Professor in the Department of Biology at the University of Massachusetts, Amherst, where he studies the development and evolution of complex morphologies, using the craniofacial skeleton in bony fishes as his main experimental model. Dr Albertson was awarded his PhD from the University of New Hampshire in 2002 for a thesis entitled Genetic Basis of Adaptive Morphological Radiation in East African Cichlid Fishes. He then went on to carry out postdoctoral research on zebrafish developmental genetics at the Forsyth Institute and Harvard School of Dental Medicine. He won the Ernst Mayr Award in Evolutionary Biology, is an NSF CAREER award grantee, has been the Keynote or Invited speaker at numerous conferences, and received the College of Natural Sciences Outstanding Teacher Award in 2016.

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## FUNDING

NSF

NIH

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## COLD AS ICE: ANTIFREEZE PROTEINS IN POLAR FISHES

**Professor Arthur DeVries** of the University of Illinois is the discoverer of anti-freeze proteins in polar fishes, which enable them to survive and thrive in polar environments, where they would otherwise freeze to death. This unique adaptation forms a cornerstone of the polar ecosystem and biodiversity, as a host of other species are reliant on these fishes as a food source.

### Polar environments: extreme temperatures and unique adaptations

Organisms in the Polar Regions are highly adapted for survival right at the lower end of the thermal range of our planet. Survival in such conditions requires a variety of specialised biological attributes, in both the land and marine creatures that inhabit and thrive in these areas. Endothermic animals, which can generate their own body heat through metabolic processes, tend to develop layers of insulatory tissue to maximise the efficiency of their metabolism. Examples include the blubber layers of whales and Polar Bears. However, ectothermic animals, such as fishes, which rely on the external environment to regulate their body temperature, face a significant challenge to generate enough energy for growth and for routine activity. Some fishes can avoid freezing by living in deeper waters in a supercooled state, thereby avoiding

contact with ice crystals that could enter their body and grow, causing them to freeze. However, for those that live in shallower waters and in particular those that exploit ice structures as specialised habitats that offer shelter from predators, exposure to and ingestion of ice crystals is inevitable. Therefore, for such fishes, some biochemical cunning is required to prevent their bodies from freezing in the frigid polar oceans.

Polar oceans are cold all year-round, but unlike the air over polar landmasses that can fall to incredibly low temperatures, they rarely fall below their freezing point of  $-1.9^{\circ}\text{C}$ , as a consequence the high heat capacity of water and the insulating ice cover. Nevertheless, polar water temperatures are sufficiently low to freeze poorly adapted organisms. Despite the challenges to survival and success posed by the polar marine environment, a plethora of species



thrive and reproduce here. The area most studied by Professor DeVries is called the Ross Sea, which is located in Antarctica. This area harbours a wide variety of species including approximately 100 species of fish. This includes the aptly named Antarctic Toothfish, which can grow up to a fearsome two metres long, demonstrating that cold water is no obstacle to the growth of ectotherms, provided they are suitably



adapted. Other animals that call this area home include penguins, seals and whales. However, ecologically speaking, one of the most important families of animals in this area is the notothenioids. The notothenioids represent the dominant family of Antarctic fish. They have evolved to fill a huge variety of niches and are found at a range of depths within this aquatic environment, despite lacking swim bladders, which enable fish to control their buoyancy. They provide an essential source of food for a significant number of other species and are fundamental to the biodiversity of this area.

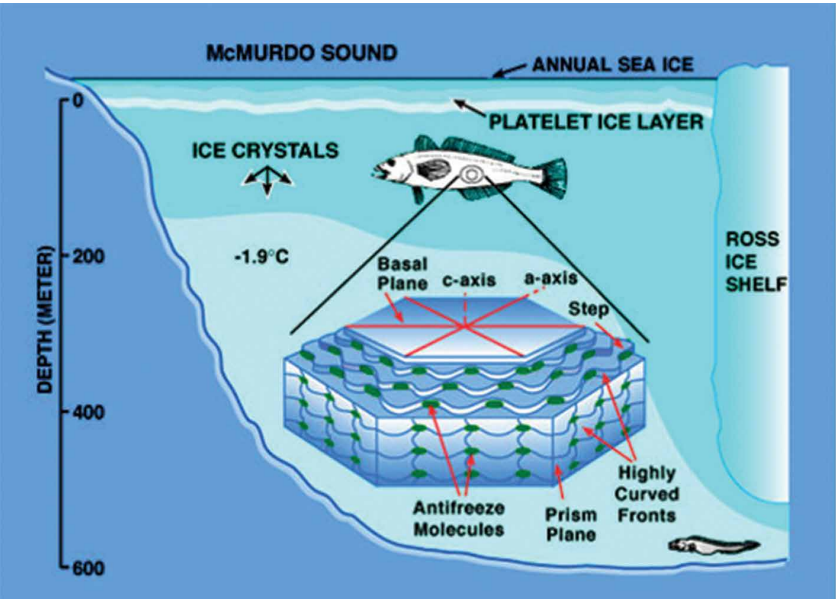
A significant key to the success and versatility of the notothenioids is their ability to resist becoming frozen at very low temperatures. This is in large part due to their production of antifreeze proteins, which inhibit the growth of ice crystals in their bodily fluids. Professor DeVries tells Scientia of the importance of this adaptation for biodiversity in the region: ‘If it were not for the capability of polar fishes to avoid freezing – as a result of the presence of blood antifreeze proteins – the polar ecosystems would be quite different. Many polar birds, seals and whales prey upon the polar fishes and they make up a large portion of their diet. If the fishes were absent then there would only be invertebrates as a source of prey and the diversity of the bird and seal fauna would be much reduced.’

Professor DeVries has devoted his career to studying how these proteins function to permit survival at extremely low temperatures. In this article, we take a look at his fascinating research career and some of his key results and motivations. His work has involved challenging research in remote Polar Regions to catch fish samples and determine how their evolutionary adaptations in antifreeze proteins make them uniquely suited for life in the freezer.

**Professor DeVries’ discovery of antifreeze proteins in the late 1960s**

Professor DeVries discovered antifreeze proteins while working as a young researcher in the 1960s. He was based on the shore of McMurdo Sound on Ross Island at McMurdo station, a major Antarctic research base run by the United States Antarctic program. Here, he tells Scientia how he came to be involved in this type of research and what he enjoyed about it: ‘I heard about a research position in Antarctica with a professor from Stanford University and applied and spent 13 months at McMurdo Station, Antarctica in 1961

**‘How the antifreeze glycoproteins, as well as other antifreeze proteins, evolved into functional antifreeze molecules is of much interest to evolutionary biologists’**



performing an investigation of the respiratory metabolism of the endemic notothenioid fishes caught in the Sound. I enjoyed the combination of rigorous field work (catching the fish) and laboratory work in this remote location.’ He went on to explain how he made his fortuitous discovery of antifreeze proteins: ‘During these experiments I noticed that a deep water notothenioid fish would freeze to death if any ice was present in our refrigerated salt water while those caught in the shallow water survived in the presence of ice. I decided to investigate why there was a difference in these species living in water of the same temperature (-1.9°C) for my PhD thesis research at Stanford. I investigated what compounds were responsible for their capability to avoid freezing in this environment while fishes in temperate waters would freeze to death at -0.8°C. My study culminated in the discovery of the antifreeze glycoproteins, the compounds responsible for their extreme freeze avoidance.’

In his early characterisation of this extreme freeze avoidance, Professor DeVries showed that the freezing point of the serum of such fish was -2°C, which was well below the freezing point of the serum of fish that inhabit warmer waters. Additional salt

content in the serum of the cold-water fish could only explain a portion of this difference and the balance was largely because of the presence of antifreeze glycoproteins.

This discovery of antifreeze proteins has opened up a completely new field of research into biological antifreezes, with labs all over the world now investigating these proteins, their occurrence in nature and use in biotechnological processes.

**Current knowledge and applications of antifreeze proteins**

In the years following the initial discovery of antifreeze proteins, significant research has been devoted to identifying new types of these proteins and characterising their mechanism of action, which is not yet completely understood. However, in general terms, the current accepted mechanism of action of antifreeze proteins involves essentially irreversible binding (through adsorption) to the sites of growth of the crystal faces of ice crystals. This results in the antifreeze proteins dividing the ice surface into many small domains whose growth fronts become highly curved. Such fronts would have a higher surface free

**‘My study culminated in the discovery of the antifreeze glycoproteins, the compounds responsible for their extreme freeze avoidance’**



energy than a straight growth front. For growth to occur, energy must be removed from the system, which is accomplished by lowering the temperature. This is the same thing as lowering the freezing point. Probing the ice/water interface indicates that this is a viable hypothesis. Antifreeze proteins are an order of magnitude more effective (on a molal basis), when compared with automobile antifreezes such as ethylene glycol, which operate via the mass action effect, and hence aren’t as effective in preventing ice formation. Antifreeze proteins have been of immense interest to scientists in the years since their discovery. Professor DeVries tells Scientia about their impact in the fields of biochemistry, evolutionary biology and physical chemistry: ‘In the field of biochemistry it is of great interest how proteins interact with water. The fact that the antifreeze proteins can recognize the solid phase (ice) in the presence of an excess of water molecules is amazing. How the antifreeze proteins recognize the ice and preferentially bind to it is of interest to physical chemists and biologists alike. The details of exactly how the bound antifreeze proteins prevent the growth of ice remains to be elucidated,’ he explains. ‘In the area of evolution, the antifreeze proteins are an excellent example of convergent evolution as the antifreeze glycoproteins in the Antarctic notothenioid fishes are identical to those in the unrelated Arctic cods but they have different evolutionary origins. How the antifreeze glycoproteins, as well as other antifreeze proteins, evolved into functional antifreeze molecules is of much interest to evolutionary biologists.’

Biological antifreezes also have a wide range of potential applications in biotechnological industries. The ability to prevent freezing using biologically compatible agents, has inherent value, and the production of highly potent synthetic antifreezes in the lab, is the goal of modern research. Professor DeVries mentions some of the potential future uses: ‘There is interest in creating synthetic compounds which mimic the antifreeze compounds in fishes but have greater antifreeze activity with the end point being able to prevent freezing of cells and tissues well below their natural freezing points. Such compounds might be useful for storing organs for transplantation as well as preventing deterioration of certain foods during cold storage.’

**Antifreeze proteins: won’t freeze, won’t melt**

The latest development in this research has been the somewhat surprising discovery that antifreeze proteins also prevent ice crystals from melting. This more recent research was carried out in collaboration with Dr Paul Cziko of the University of Oregon and Professor Chi-Hing Cheng of the University of Illinois. The team found that ice that had been stabilised through binding with antifreeze proteins resisted melting and would not melt until exposed to much higher temperatures than expected. This was true both in the laboratory and also in living fish specimens. It is thought that the ice crystals present in polar fishes arise from their external environment and are introduced into their bodies through processes such as feeding or water entering their gills. It had been previously observed that the bodies of polar fishes contained ice crystals in locations such as their blood and spleen. It was unclear if the fish somehow eradicated these crystals or if they melted during summer warming periods. The team monitored water temperatures in Antarctica for about ten years and concluded that the water temperatures never got high enough to cause the ice crystals inside the fishes to melt, especially given that they were stabilised with antifreeze proteins. Some Arctic fishes, in contrast, encounter seasonal warming, and so do not require antifreeze proteins year-round. Fascinatingly, these fishes degrade their antifreeze proteins in the summer to conserve energy, to build up their reserves again in the autumn in preparation for the cold winter.

However, for fishes in the colder Antarctic climate, with a year-round complement of antifreeze proteins it is currently unclear if they are able to eradicate the protein-stabilised ice crystals from their bodies. If not, and they continue to accumulate for the entire lifetime of the fish, it is unknown if they result in adverse effects, such as blockage of blood vessels or production of inflammatory reactions. If so, then the unique adaptation that enabled such fishes to thrive in this hostile environment, antifreeze proteins, could also come with a down side, as they appear to contribute to accumulation of the crystals. This question of ice crystal accumulation and eradication is the current focus of Professor DeVries’ research. He believes that there must be a method that the fishes use to eradicate the crystals: ‘The fishes must have a biological mechanism for ridding themselves of these ice crystals because if they continue to accumulate them, one would assume that the ice burden would become so great that it would overwhelm their physiological systems. Thus, how do the fishes avoid accumulating detrimental levels of ice’. How indeed?





Credit: L. Fields

# Meet the researcher

**Professor Arthur DeVries**  
Department of Animal Biology  
University of Illinois,  
USA

Professor Arthur DeVries is an Emeritus Professor in Animal Biology and Molecular and Integrative Physiology at the University of Illinois. Professor DeVries has devoted his career to the discovery and characterisation of antifreeze proteins present in the blood and tissues of polar fish. He was the first person to discover these proteins, and their discovery opened up a completely new field of research. Professor DeVries was elected a Fellow of the American Association for the Advancement of Science in 1984, for his distinguished research on biological antifreeze compounds of coldwater fishes. He has been presented with many awards, including the Premio Internazionale ‘Felice Ippolito’ (international prize for biological excellence) by the Italian the Accademia Nazionale dei Lincei and National Antarctic Programme (2005), the Life Time Achievement Award for discovery and research on antifreeze proteins in polar fishes at the 1st International Ice-Binding Protein Conference. Finally, he also caught a previously unknown Antarctic fish, which was described by the famed Russian Ichthyologist, Dr A. P. Andriashev and named ‘Paralaporis devriesi’.

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## FUNDING

Division of Polar Programs, Geosciences, The National Science Foundation

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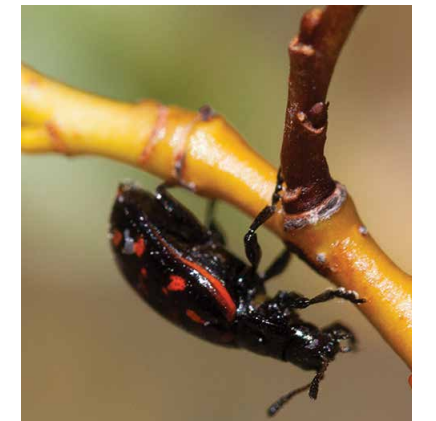
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## EXPLORING EVOLUTION BY STUDYING BEETLES LIVING ON THE EDGE

**Professors Nathan Rank and Elizabeth Dahlhoff**, along with their collaborators and students, explore the question of evolutionary responses to changes in climate by studying a small, native beetle that lives two miles high in the mountains of Eastern California.



### Genetic Response to Climate Change?

Throughout Earth’s history, massive climate shifts have led to huge changes in flora and fauna, sometimes very quickly. For example, the Triassic-Jurassic extinction around 200 million years ago resulted in the loss of half of all species – plants and animals – living on the planet at that time. As a result, dinosaurs rose to prominence. The later Cretaceous-Paleocene extinction wiped out three-quarters of the Earth’s plants and animals, including those very dinosaurs. The point to remember is, when massive climate changes like these occur, while many species die, some actually adapt and flourish in this new and different world. How does that happen?

Most people would offer the classic Darwinian sound-byte – ‘survival of the fittest’ – in answer to the question of species survival after a massive change in climate. Bring up ‘survival of the fittest’ to a biologist, however, and you open the door to a rich

discussion of genomics, species diversity, and adaption. These are all very important and relevant to help us understand how animals are going to survive (or not!) in environments dominated by the presence of humans. This is what biologists Professor Nathan Rank, Professor Elizabeth Dahlhoff, and their like-minded colleagues have spent over two decades trying to answer – by studying a tiny beetle that lives on the edge of its range in the snowy environs of the mountains of the American West. That beetle, according to Professor Rank, allows them to ‘study genetic and physiological mechanisms by which natural populations cope with environmental stress in a region that is experiencing significant effects of changes in climate.’

### A Beetle Living on the Edge

The Sierra willow leaf beetle – *Chrysomela aeneicollis* – is a small insect that lives on the leaves and at the base of willow trees in the high altitudes of the snow-capped

American Sierra Nevada mountains in California, and in and other cool, moist areas across the Western North America. What makes this beetle so interesting to Professors Rank and Dahlhoff is that it must survive in an extreme environment that sometimes changes dramatically from year to year. ‘So many things are interesting to me about these beetles,’ Professor Dahlhoff explains. ‘First, in the parlance of the trade, these guys are “extremophiles”, meaning they tolerate environmental conditions that would kill most other organisms. They live at very high elevations in a relatively dry place that is hot during the day and cold at night even in the summer. Then in winter, it is cold and snowy, or dry and cold. How do they survive that?’ That’s a good question.

Normally these willow beetles spend over half their life cycle in winter conditions, and so are vulnerable to winter cold. Studies have also shown that its survival can also suffer when the beetle is exposed to natural



## ‘I love that I get to explore biochemical and physiological adaptations to temperature in this really neat natural system, and I never get tired of doing fieldwork’ – Professor Dahlhoff



cold events during the summer. Recently Professor Rank, Professor Dahlhoff and their co-workers reported on the microhabitat the beetle encounters in the winter and recorded ways it adjusted to the cold in all its life stages. They also used temperature sensor data to look at the effect of snow cover and microhabitat buffering on survival during the winter, as if the beetle survives in an igloo of snow when the weather is bitterly cold.

What the team found was that the beetle's tolerance to cold varies with its life stage, and this in turn is correlated with the beetle's microhabitat temperature. The beetles are more susceptible to cold in the larval stage, and more resistant to cold in the adult stage. They showed that hemolymph – a beetle's 'blood' – is more concentrated during the winter phase than during the summer. This is in large part due to elevated hemolymph glycerol – natural antifreeze. Normally, adult beetles spend the winter in an almost hibernation state, primarily in leaf litter near their willow plant hosts. But they suffer high mortality if early, unseasonable cold keeps them from entering this stage on time.

The team's data indicate that cold tolerance is highly correlated with life stage and so beetle survival may become difficult as the climate becomes more unpredictable. In other words, if a changing climate – a run of colder or drier winters or shorter summers – uncouples the cold tolerance due to the beetle's life stage and surrounding temperatures, the beetle's survival

mechanism doesn't have time to work. If the weather gets too cold too early, the beetle doesn't get to tank up on its antifreeze in time, and can't hibernate correctly, and dies. Living high in the snowy mountains places the willow beetles in danger from just small changes in the annual winter temperatures and duration. But the biologists found something even more interesting than this – different beetles have different cold-endurance capabilities, depending on the type of a couple of key genes that they have.

### Can Rapid Climate Change Induce Evolution?

Some years ago, the team conducted a study investigating changes in gene frequency in areas of the willow beetle's DNA that code for three enzymes, isocitrate dehydrogenase (Idh), phosphoglucose isomerase (Pgi) and phosphoglucomutase (Pgm). They looked at the difference in gene frequency between the years of 1988 and 1996 in beetles in the Bishop Creek region of the Sierra Nevada mountains. Beetles high in these mountains often experience hot daytime temperatures – over 32°C – and extremely low night-time temperatures – less than -5°C – even during summer. Bishop Creek weather station data showed that weather had been unusually dry before 1988, but was then cool and wet during the years before the 1996 specimen collection.

They found consistent changes in the frequency of genes producing Pgi – an

elevation of 11% on a version of the gene called Pgi 1. However, there was no change in genes coding for Idh or Pgm. Also, they found that the response to high heat depended on the specific Pgi version. Pgi 1-1 individuals – beetles with two Pgi-1 genes (in other words, homozygous for Pgi-1) – produced a protein called HSP70 at lower temperatures compared with beetles with Pgi version 1-4 or 4-4. In addition, Pgi 1-1 individuals showed higher levels of HSP70 after raising their temperature in the laboratory in the same way as in the wild. Survival after night-time lab exposure to sub-zero temperatures depended on beetle sex, prior cold exposure, and variety of Pgi. Females produced higher levels of HSP70 than males after heat exposure, and recovery by female Pgi 1-1 homozygotes after exposure to cold was better than beetles with Pgi 1-4 or 4-4. This informed Professors Rank and Dahlhoff that the cooler climate of the mid-1990s appears to have caused an increase in the frequency of the Pgi-1 gene, perhaps due to a stronger physiological response to cold by Pgi 1-1 and Pgi 1-4 beetles. Professor Dahlhoff tells us that 'there is evidence of natural selection at Pgi, which seems to be one of the few "magic genes" of biochemical adaptation.' It appeared that the beetle evolved biochemically and became more resistant to cold because of a change of environment. And that change occurred over only eight years. Scientifically, it looked like this protein had a lot to do with it.



## ‘Elizabeth’s scientific background is quite different from mine, but we have pursued this collaboration for 20 years now and so our areas of expertise are quite complementary’ – Professor Rank

### Evolving by Protecting Enzyme Systems

Heat shock proteins are a family of proteins produced by cells when exposed to stressors, such as heat, cold, starvation, injury, exposure to toxin or infection. In the willow beetles, Professors Rank and Dahlhoff have demonstrated that the heat shock protein HSP70 is produced in response to both heat and cold stress. HSP70 acts like heat shock proteins usually act, by protecting vital proteins from damage or deactivation by cold exposure. Clearly, HSP70 is vital to the beetle's survival in extreme heat and cold, and increased levels of HSP70 seem to be a survival advantage.

In fact, the team has reported that variations of the PGI gene were related to physiological functions such as HSP70 production, heat tolerance, running speed, and metabolic rate. Correlations between PGI and female egg production, both in the lab and in the wild, indicated that these physiological responses to temperature produce changes in reproductive success among beetles with different PGI variations. Amazingly, they found that PGI frequencies fluctuated within a single generation. This means that differences in reproductive success and survival may cause pretty rapid changes in the genetic makeup of wild populations.

Finally, changes in beetle numbers during a ten-year California drought, from 1998 to 2007, showed that the range of the bug has moved upward to higher elevations, in keeping with the changing climatic conditions of the Sierra Nevada mountains. And it is happening again, with the current drought going on in California. Beetle populations have 'crashed', especially at low elevation.

Along with the data from the field fecundity experiments, beetle population numbers and abundance, the team also analysed beetle populations from different regions of the mountains. According to DNA analysis, these different beetle populations – though the same species – have actually been separated for thousands of years. Data from these different populations told these researchers that beetles living in one specific area could be more resistant to environmental stress than those living in neighbouring areas. This difference between separate populations indicated that the beetle population as a whole has enough genetic variability to respond evolutionarily to a severe change in climate overall. However, small-scale changes in weather conditions can cause local extinctions at warmer, drier sites and at lower elevations, causing the beetles to migrate to higher elevations. According to

Professor Rank: 'We have identified genes involved in metabolism and responses to stress and quantified their effects on survival and reproduction of insects in the mountains of California; a region where annual variation in rain, snow, and average temperatures is high.' These findings are evidence that evolution is going on right before our eyes in a complex organism, not some single celled bacterium in a Petri dish. The implications here for scientific understanding of evolution and adaptation to climate change are profound.

### The Work Continues

Professors Rank and Dahlhoff have methodically investigated the willow beetle for decades. 'Our most recent work shows that genes on the mitochondrion interact with genes from the nucleus to influence responses to environmental stress. It also appears that responses to low oxygen (hypoxia) interact with responses to stressful temperature to influence beetle populations in the wild. This has been a real surprise, because until now, most physiologists did not think a small insect would even be limited by environmental hypoxia. Turns out, beetles are just like humans in this respect – they can get 'out of breath' at high elevation. Our current work aims to understand how populations survive both summer and winter thermal stress and environmental variation,' Professor Rank tells us. To that end they hope to continue to investigate the selective pressures on the beetle during severe winters, to look at how cold and energy stressors interact to shape the beetle's physiological performance during winter and the subsequent growing season.

'We will complete our work on interacting stresses of temperature and oxygen supply on physiological and evolutionary responses to a changing environment,' Professor Rank explains. 'We plan to achieve this by investigating populations in the winter time and in the summer.' The team's work on winter survival and responses to stress will be invaluable for studying environmental effects on population persistence. In other words, how does a population adapt and survive – and evolve – in the face of severe changes in climate. A beetle surviving at its climate limit may offer us clues for how we can survive our current global climate disaster.





## Meet the researcher

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Professor Nathan Rank received his B.A. in Biology from Kalamazoo College, Kalamazoo, Michigan, in 1983. Thereafter he pursued doctoral studies in Ecology and Evolutionary Biology at the University of California at Irvine and received his Ph.D. in 1990 from UC-Davis. Professor Rank then took a post-doctoral fellowship at the Laboratory of Animal Biology of the Free University of Brussels, Belgium, and the Zoological Institute of the University of Basle, Switzerland, followed by an assistant professorship at the Group of Experimental Ecology of the Swiss Federal Institute of Technology in Zurich. In 1995, he joined the faculty of the Department of Biology of Sonoma State University, where he is now Professor of Biology. Professor Rank's research interests include local adaptation and host plant specialisation in insects, which involves fieldwork, biochemical studies, and population genetic approaches to the issues.

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Professor Elizabeth Dahlhoff received her B.A. in Biology and Chemistry from the University of California-Santa Cruz in 1986. She then received a graduate research fellowship at the Scripps Institution of Oceanography from 1987 to 1992, receiving her Ph.D. in Marine Biology from Scripps and the University of California-San Diego in 1993. She was then the Andrew Mellon Postdoctoral Fellow in the Department of Zoology at the Oregon State University until 1995, when she joined the Department of Biology of Sonoma State University as an assistant professor. In 1997 she joined the Department of Biology of Santa Clara University, where she is now Professor and Chair of the Biology Department. Professor Dahlhoff's research interests focus on elucidating genetic, biochemical and physiological mechanisms by which animals respond to environmental change. She is especially interested in how animals survive in extreme environments.

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### FUNDING

National Science Foundation  
White Mountain Research Center  
Santa Clara University  
California State University Program for Education and Research in Biotechnology  
Wenner-Gren Foundation, Sweden  
California Desert Research Fund



## SOCIAL BEHAVIOUR IN BIRDS: WHAT MAKES THEM TICK?

**Professor Elizabeth Adkins-Regan** of Cornell University investigates the physiological mechanisms underlying social and reproductive behaviour in birds, including the life-long bonds formed by monogamous pairs, parental behaviours and the influence of parents on offspring behaviour. Her team studies the hormones and neurohormones expressed by birds, to gain a fundamental understanding of these processes.

### No man is an island: social interaction and its basis

A multitude of species demonstrate social behaviours. These behaviours confer inherent advantages. For example, wolf packs hunt cooperatively and prairie dogs can keep watch for predators and alert others to their presence. As a species, humans demonstrate some of the most complex social interactions. Our ability to communicate complex concepts, and to store and share knowledge in the form of the written word, is the basis upon which our global civilisation is built. From the social dynamics of family units, which function to produce and rear children, to the way countries are governed and interact with

each other, our society is based on social hierarchies and networks. How these social bonds arise and function, at a physiological level, is incompletely understood, both in humans and in the animal kingdom. By studying the physiology of the interactions of specific animals, we can determine effects that are applicable across numerous species, including humans. Professor Adkins-Regan, who is a hybrid between an animal behaviourist and a neuroscientist, explains the applicability of her results to other species for Scientia: 'While the goal of the research is not to understand the human species, it does help provide the broader biological context for understanding our behaviour and its pathologies. For example, problems such as dysfunctional romantic/

sexual relationships or poor parenting are all too frequent and have serious health and economic consequences.'

However, studying social interaction in animals is not just of value because of its potential applicability to understanding human behaviour. This research is also of inherent interest and value in its own right, when employed in specific animal species, to increase fundamental knowledge about their behaviour. Professor Adkins-Regan explains the fundamental nature of the research she undertakes and her appreciation of the people who make it happen: 'The research that I do is basic, or foundational, research, in which knowledge is sought in order to increase understanding of the world around



us. What matters to me personally is to help people understand the value of basic, foundational (“blue sky”) research, the importance of funding such research (taking the long view and not just funding for short-term impact), and the value of the graduate students and post-docs whose creativity and effort is absolutely essential for scientific progress. I am immensely grateful to the US National Science Foundation for promoting and upholding these values.’

Increasing fundamental knowledge of animal behaviour is not merely an academic exercise, but plays an important role in safeguarding biodiversity and fragile ecosystems. We currently live in a time of unprecedented loss of biodiversity and extinction. In fact, we are currently in the midst of a mass extinction event, and human activity is thought to be the primary cause. It is the hope of Professor Adkins-Regan that by understanding the social interactions of different species a little better, we might be more invested in protecting them from extinction, lest we lose something before we have even begun to fully understand its complexity and value: ‘We share the planet with an enormous number of kinds of animals, whose lives and behaviour need to be understood for humans to appreciate them sufficiently to care about their futures.’

So what do the team study, specifically? As Professor Adkins-Regan explains: ‘The Nobel-prize winning scientist Niko Tinbergen pointed out that to understand the behaviour of an animal, you need to figure out its adaptive function, evolutionary history (phylogeny), development (ontogeny), and physiological and other mechanisms. My contributions have ranged across all four categories but have mainly focused on mechanisms. Animals have a fascinatingly diverse array of highly adaptive social and mating arrangements, and figuring out the mechanisms responsible for those is an exciting challenge.’ In the remainder of this article, we will focus on the main themes in Professor Adkins-Regan’s work – monogamous pairing in birds, parental care in birds, and maternal effects in birds.

**Monogamous pairing in birds**

Professor Adkin-Regan’s research has focused on the tendency of some bird species to form life-long monogamous pairs. She tells Scientia why birds are useful in such research: ‘Doing this work in birds is important because birds are the largest

**‘What struck me and motivated bringing this research theme into my lab is that, although birds are overwhelmingly the most monogamous vertebrates, they had been almost completely neglected in the study of hormonal and neural mechanisms for pairing’**



group of terrestrial vertebrates and their lives in the wild are the best studied of all animals.’ Previous work by other researchers on the neuroendocrinology of pair bonding had been performed in a monogamous rodent called the prairie vole. This work showed that the neuromodulator dopamine and nonapeptides, which are neuropeptides that derive from the vasopressin and oxytocin families of hormones, are involved in the physiology of pair bonding. Professor Adkins-Regan realised that birds remained understudied in this context: ‘What struck me and motivated bringing this research theme into my lab is that, although birds are overwhelmingly the most monogamous vertebrates, they had been almost completely neglected in the study of hormonal and neural mechanisms for pairing.’

To date, the majority of the work performed by the team has used the zebra finch, and they have made some interesting findings thus far. In 2006, in work performed by Dr Michelle Tomaszycski, a former postdoctoral researcher in the group, they found that oestrogens and androgens, also called ‘sex hormones’, are not required for successful monogamous pairing between zebra finches, suggesting that other mechanisms are at play. More recently, Sunayana Banerjee, a former graduate student in the group, found that when birds are starting to pair, they release much more of the neuromodulator dopamine, compared with when they are

participating in social behaviours unrelated to pairing. This result suggests that dopamine may be heavily involved in regulation of pairing behaviour.

Finally, the lab is responsible for establishing a new animal model to study monogamous pairing in birds. It is the first to compare the pairing behaviour of the king quail, a monogamous bird, to that of the closely related, albeit less monogamous, Japanese quail. Adkins-Regan concluded that the king quail/Japanese quail model could form a new comparative system. Comparing the pairing behaviours and associated physiology of the birds could enable robust conclusions to be drawn about the nature of monogamous pairing, whereby there is a non-monogamous control result for each measured parameter in the monogamous animals. Zebra finches do not have a closely related non-monogamous bird and so there was a need for a new comparative model. The new system will allow researchers to robustly test hypotheses about mechanisms underlying behaviour. Professor Adkins-Regan told Scientia about her hopes for the model: ‘I am very excited about the comparison between the two quail species. Both are easy to house and work with, and the contrast in their behaviour is a potential goldmine. There is so much that would be important to do that I hope other researchers also recognise their potential and decide to start working with them.’



**Parental care and maternal effects in birds**

Invariably, the functional outcome of monogamous bonding is the production and rearing of offspring. In fact, in many monogamous species, caring for young is undertaken in a biparental fashion, whereby both male and female animals divide the required work in an equal fashion. This is true of zebra finches and the team have studied the endocrinology underlying this cooperative behaviour. Their current hypothesis is that prolactin, a hormone produced in the pituitary gland, regulates this form of parental behaviour. Prolactin has already been shown to be involved in parental behaviours in fishes and mammals and also in doves, but very little was previously known about its role in parental behaviours in songbirds such as zebra finches. The results so far appear to support a prolactin hypothesis of parental regulation in zebra finches.

The team are also interested in maternal effects in birds – that is, the effect that parents have on the behaviour of their offspring, outside of direct genetic inheritance. In birds, a good example of this is the potential for large quantities of hormones to be present in the yolk of eggs, thereby providing a mechanism where maternal hormones can influence the behaviour of the embryo when it reaches adulthood. However, parental behaviour also has significant effects on the behaviour of their offspring. The team have studied some aspects of this. They have looked at the behaviour of zebra finches that were raised by a father alone, and did not have the experience of parenting from both male and female birds. The chicks undergo normal development, with the exception that as adults they demonstrate abnormal stress hormone response systems. In the case of male chicks reared under such conditions, they prefer to form bonds with other males when they reach adulthood. This is unusual in zebra finches, which ordinarily form mixed sex pairs.

The team have studied the neuroendocrinology of the chicks themselves, and how this influences the effect their parents have on them. For example, they have found that nonapeptides of the

vasopressin family play an important role in how young male zebra finches learn from their fathers. Under normal conditions, young male zebra finches learn songs from their fathers through a process of social interaction. The team injected young male hatchlings with agents that promoted or suppressed nonapeptide signalling. They then monitored the quality of the learned songs when the birds reached maturity. Birds with enhanced nonapeptide signalling demonstrated better quality songs that were a better match to their tutor’s songs, compared with birds with suppressed signalling, demonstrating that nonapeptides are important in social interaction and vocal learning at an early age.

**‘Doing this work in birds is important because birds are the largest group of terrestrial vertebrates and their lives in the wild are the best studied of all animals’**

**Future work**

Professor Adkins-Regan spoke with us about what direction she would like her research to take next. This includes an exploration of how zebra finches can take new partners if their original partner dies: ‘There are still many unanswered questions about mechanisms for pairing in the zebra finches. For example, the pair bonds are permanent, and birds only form a new pair relationship if the partner dies. Sudden loss of the partner activates a hormonal stress response. I would very much like to know whether and where that hormonal change acts in the brain to open up the “window of opportunity” that enables the survivor to quickly change to a state of willingness to form a new pair relationship.’ She would also like to further explore the prolactin hypothesis of parental care in zebra finches: ‘Another priority is to carry out the critical manipulation experiments to determine whether prolactin really is a basis for parental behaviour in the zebra finches or in songbirds more generally. I am always bothered when the literature assumes something about hormones and behaviour that has never been properly tested.’





# Meet the researcher

**Professor Elizabeth Adkins-Regan**

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Professor Elizabeth Adkins-Regan is a behavioural neuroendocrinologist based at the Department of Psychology and Department of Neurobiology and Behavior at Cornell University. Professor Adkins-Regan has devoted her career to studying the physiological basis of social and reproductive interaction in animals. In particular, she is interested in understanding the basis for the life-long monogamous bonds formed by some birds. Her research group conduct most of their research in birds, including the zebra finch and two species of quail. Recently, she was awarded the Howard Bern Award from the Society for Integrative and Comparative Biology and is the author of a book, and numerous book chapters and research articles on this topic.

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## FUNDING

US National Science Foundation: IOS-1501336, IOS-1146891, IOS-1310908 and IBN-0130986

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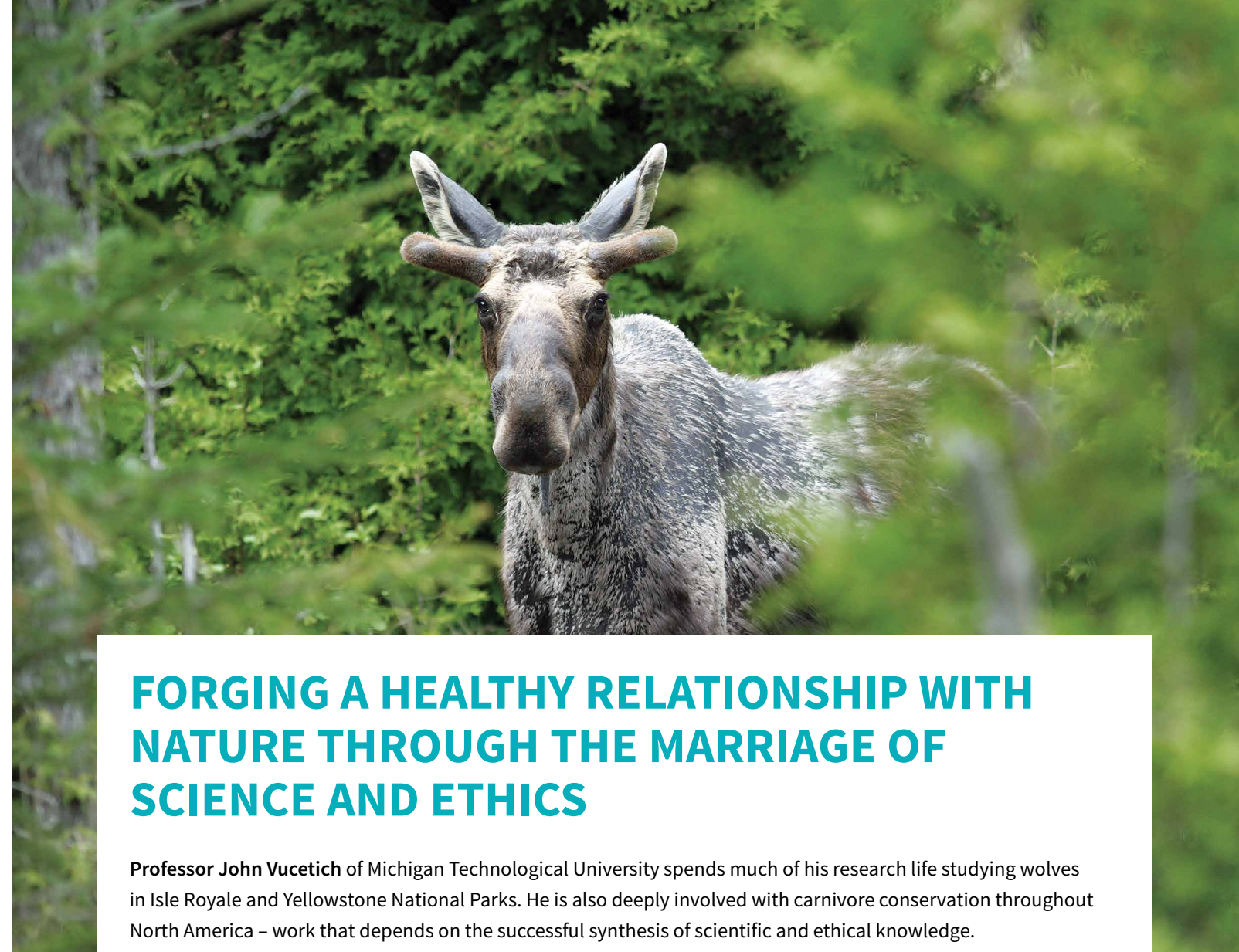
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## FORGING A HEALTHY RELATIONSHIP WITH NATURE THROUGH THE MARRIAGE OF SCIENCE AND ETHICS

**Professor John Vucetich** of Michigan Technological University spends much of his research life studying wolves in Isle Royale and Yellowstone National Parks. He is also deeply involved with carnivore conservation throughout North America – work that depends on the successful synthesis of scientific and ethical knowledge.

### The Isle Royale Project – a Source of Wonder

Isle Royale is a remote island protected by the frigid waters of Lake Superior, the largest freshwater lake in the world. The island has been inhabited by a single species of large predator, the wolf, and their sole prey, the moose. That predator-prey system is globally distinct for being protected from hunting and persecution and for being supported by a forest protected from logging. The wolf and moose populations have been studied intensively for nearly six decades, representing the longest running study of any predator-prey system in the world. Professor Vucetich has been leading that project, with Professor Rolf Peterson, since 2000. Much of Vucetich's work has focussed on developing knowledge about predation ecology and population genetics.

A keystone for understanding predation, on the whole, is to understand the dynamics that underlie kill rate – the frequency at which predators kill their prey. Ever since the predator-prey equations of Lotka and Volterra, ecologists had believed that kill rate depends essentially on the number of prey. The rationale is robustly simple – predators kill more frequently when their prey are more abundant because they are easier to find. The idea also seemed to be supported by significant empirical evidence. Other factors – such as weather and the health of individuals in the prey population – were certainly important, but the essence is prey abundance. An alternative idea of heretical proportion was introduced in the 1990s: kill rate might depend not so much on prey abundance, but more so on the ratio of prey to predator. The idea was tough to accept for several critical theoretical reasons (for example, if kill rates are ratio dependent,

then predators are less likely, in theory, to create trophic cascades). Controversy was further fuelled by a relative dearth of empirical evidence capable of evaluating ratio dependency. Vucetich showed that kill rates by Isle Royale wolves are unquestionably better explained by the ratio of predator to prey. That demonstration paved the way to ratio-dependency becoming a conventional idea.

Vucetich also used observations of Isle Royale wolves to resolve another kill rate mystery. Behavioural ecologists studying social carnivores – wolves, lions, African wild dogs, and the like – had long been perplexed by the tendency for the per capita kill rate to decline as group size increases. That pattern ought to lead to the dissolution of larger social groups, because predators would tend to do better foraging on their own, or perhaps with just one partner. Vucetich demonstrated



# ‘I wanted to contribute to a better relationship between humans and nature. I naively thought the need for more scientific knowledge was the limiting factor in making for a better relationship.’



in a 2004 *Animal Behaviour* article and later summarised:

‘Wolves living in larger packs each get more food because they lose less food to scavenging ravens. They do this by eating a moose so quickly that ravens have less time to scavenge. The details are fantastically complicated, and while wolves in larger packs must share food among their brothers and sisters, parents and offspring, that sharing is not so costly as losing food to scavengers. So ravens have something to do with explaining why wolves live such intensely social lives—a trait that is otherwise rare among carnivores. What an astonishing connection.’

Observations from Isle Royale by Vucetich’s team have also deepened our understanding of a potentially valuable conservation tool – genetic rescue, which occurs when the introduction of one or more unrelated individuals into an inbred population mitigates the detrimental effects of inbreeding. In 1997, a wolf immigrated to Isle Royale by crossing an ice bridge that formed during an unusually cold winter. The severely

inbred condition of Isle Royale wolves and the beneficial effect of the immigrant were revealed by genetic analyses demonstrating that, within three generations, more than half of the population’s ancestry was attributable to the immigrant. But the demographic benefits of that powerful genetic rescue were muted by the moose population (i.e., the wolves’ food supply) having collapsed in the previous year due to another severe winter. One of the general lessons of this genetic rescue event is that in a stressful environment, genetic rescue can be beneficial even if it does not result in a clear demographic response.

That genetic rescue event also reversed a decades’ long belief that the wolf population had been genetically isolated and free of inbreeding depression. Those observations also revealed an unexpected adverse impact of climate warming. In past decades, occasional gene flow had been facilitated by ice bridges that formed during most winters. With climate warming, ice bridges are now rare and will soon be a thing of the past. By 2016, inbreeding had brought the wolf population to the brink of extinction and the

moose population was showing early signs of unsustainable growth. Vucetich highlighted, in a 2016 essay in *Natural History*, how the wolves of Isle Royale represent a major, unresolved policy concern for knowing when it is right to mitigate the adverse effects of climate change in protected areas.

## Complexities revealed only through long-term research

Understanding the extent to which predators affect prey and subsequently shape ecological communities has been one of the great, perennial motivations in ecology. With respect to those dynamics, Isle Royale has exhibited four distinct phases over the past six decades. During the 1960s and 1970s, wolf predation was high, suppressing moose abundance and allowing vigorous growth of the forest. To have such a clear impression after two decades of observation would lend considerable confidence to one’s perceived understanding, especially given that few ecosystems are studied as intensively or for as long.

What can undermine such confidence is continued observation. That two-decade pattern abruptly reversed itself during the 1980s. Wolf predation plummeted, moose erupted and forest growth was impaired. That reversal was reported in a 1994 Science article and is considered the first trophic cascade to be documented in a terrestrial ecosystem.

Without warning, the pattern quickly switched again during the mid-1990s. Moose abundance collapsed, wolf predation was reignited, and the forest rebounded in a way that had not been experienced for nearly a century. Another reversal occurred in 2009. Again, wolf predation plummeted, moose populations erupted, and the forest began to endure another round of increased browsing.

These dynamics are not adequately explained by predator-prey cycles of the kind that Lotka and Volterra described almost a century ago. Lotka-Volterra dynamics are, for example, characterised by an impressively consistent tempo – like the inviolable 10-year cycle of lynx and hare. On Isle Royale, the tempo is irregular and the abrupt shifts are sparked by inherently unpredictable events. The first switch from high to low predation was triggered by a disease that struck the wolf population in the early 1980s (the disease, canine parvo virus, was inadvertently brought to the island by

humans). The second switch from low to high predation was triggered by the coincidence of rare events in 1996 and 1997 – a catastrophically severe winter whose affect was amplified by extremely high moose abundance and a wolf population reinvigorated by genetic rescue, which arose from the idiosyncratic coincidence of a winter cold enough to produce an ice bridge and an individual wolf whose dispersal was fortuitously and impressively successful. The most recent abrupt shift in dynamics occurred in 2009 when the benefits of genetic rescue unexpectedly evaporated.

Predicting the future dynamics of an ecosystem from the laws of nature has long been a unifying purpose of ecological science. Vucetich says, ‘Regularities that could be called laws of nature are certainly important for understanding ecosystems, but Isle Royale suggests a different view. Dynamics on Isle Royale have been better explained by inherently unpredictable historical contingencies, events such as extreme weather or novel diseases, than by any regular patterns.’

Vucetich also believes that admitting the dominant role of historical contingency goes well beyond the conventional understanding of environmental stochasticity. Vucetich concludes that ‘acknowledging the importance of inherently unpredictable historical contingencies on ecosystems would profoundly affect our approach to natural resource management, which depends heavily on the faith we put in our capacity to predict the future consequences of our actions toward nature.’

## Environmental science and environmental ethics

The path that led Vucetich to study wolves on Isle Royale was unplanned, fortuitous, and depended critically on support from mentors – especially Professor Rolf Peterson, who began leading the Isle Royale wolf-moose project in 1970, a year before Vucetich was born. But motivation to travel down that path, Vucetich recalls, ‘began as a young boy. I enjoyed the outdoors and had great outdoor experiences in Boy Scouts. At first, my interest was little more than satisfying a sense of outdoor adventure and later a sense of intellectual adventure. Over time my interest changed – I wanted to contribute to a better relationship between humans and nature. I naively thought the need for more scientific knowledge was the limiting factor in making for a better relationship. I eventually discovered that I had an independent interest in philosophy and environmental ethics.’

That interest in philosophy exposed Vucetich to some of the essential thoughts of western knowledge. There was the is/ought problem, developed by 18th century philosopher David Hume, which posits that understanding what counts as a right relationship with others (including nature) cannot be determined from facts alone. And, there were the complicating thoughts of the great American Philosopher, Hillary Putnam, who demonstrated that distinction between facts and values is quite a bit blurrier than is typically supposed. Vucetich says, ‘Over time, I came to believe that the best I could do to help bring about a better relationship with nature is to marry the insights of environmental science and environmental philosophy.’

## From wonderment to sustainability

Vucetich believes a critical and undervalued role of science is to inspire a sense of wonder for nature because, as he has written:

‘Think about knowledge that makes you go “Wow!” Wow, that’s so



beautifully complicated . . . magnificently nuanced . . . astonishingly connected . . . It would seem awfully difficult to intentionally abuse nature while being held by its wonder. How can you do anything but care for nature, while [genuinely] astonished by its beauty, complexity, and interrelatedness?’

But Vucetich’s argument goes further. While some argue that scientists should restrict themselves to mere custodians of fact and refrain from any further advocacy for the environment, Vucetich sees it differently. He and long-time collaborator, ethicist Michael Paul Nelson of Oregon State University, argued in a 2009 *Conservation Biology* article that scientists are citizens before they are scientists. As such, and in the spirit of playwright Henrik Ibsen, citizen-scientists have an obligation to work for that which they can argue – honestly and transparently – is right.

Vucetich has also explained, in a 2015 *Conservation Biology* article, how nature is valuable not only for human well-being, but that nature also possesses intrinsic value. That is, nature ‘deserves to be treated with . . . concern for its welfare [and] in a just manner.’ Acknowledging nature’s intrinsic value has a profound impact on our understanding of sustainability. When we fail to acknowledge nature’s intrinsic value sustainability reduces to it vulgar form, as Vucetich and Nelson called it in a 2009 *Bioscience* article. That is, sustainability is no more than ‘exploiting nature as much as we like without infringing on our future capacity to do so.’ In sharpest possible relief, acknowledging nature’s intrinsic value lifts sustainability to its virtuous form, where we ‘exploit nature as little as necessary to maintain a healthy, meaningful life.’

The future, it seems, will depend critically on our collective ability to operate wisely at the interface between science and ethics.





# Meet the researcher

Professor John Vucetich

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Professor John Vucetich is from the School of Forest Resources and Environmental Science at Michigan Technological University. After graduating from Michigan Technological University with a BSc in Biological Sciences and a PhD in Forest Sciences, he took a Research Assistant Professorship at the same institution and became an Associate Professor in 2011. He has authored or co-authored over 75 peer-reviewed articles that have been collectively cited more than 3400 times. His scientific research has led to valuable insight pertaining to predation and population genetics. Professor Vucetich has also distinguished himself as an expert in conservation ethics. That expertise is comprised of equal parts scholarly rigor and real-world conservation experience. His writings on ethics have appeared in scholarly venues, such as Conservation Biology, Bioscience, and Conservation Letters and popular outlets such as The New York Times, Natural History, Huffington Post, and The Ecologist. Writing on a wide range of topics – trophy hunting, wilderness, endangered species, sustainability, animal welfare, and climate change mitigation – Professor Vucetich’s work has been acknowledged by its influence on policy and scholarship. He has been involved with numerous documentaries, art exhibits and museum exhibits which detail the wolves and moose of Isle Royale. He has been fictionalised as the main character in a novel (Winter Study by Nevada Barr), which made the New York Best-Sellers list at #10 for hard-cover fiction.

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## FUNDING

U.S. National Science Foundation

U.S. National Park Service

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# BREAKING FREE THE STONES OF THE PAST

Professors William Hunt and Ralph Hartley are both experienced archaeologists and anthropologists within the Department of Anthropology at the University of Nebraska-Lincoln. They have recently returned from the islands of Southeast Alaska, having set out to identify the source of the mysterious human-built piles of rock called ‘cairns’ scattered over the coastal mountains.

It’s difficult to remember, as we sit in our air-conditioned offices worrying about the latest report and the cost of today’s coffee, that most of human history has been spent in close contact with the soil, the trees, the rocks – with our ancestors struggling to improve their lives by changing the very landscape they stood upon. Many of these changes are still visible today, from the terraced slopes of hillside agriculture through to fields cleared of rocks for the plough. But the thinking behind many of our ancient modifications is lost in the mists of time – who made this, why did they do it, what were they thinking?

Nowhere is this better illustrated than in the existence of cairns, literally a man-made pile of rocks. The simplicity of the materials required (rocks are quite common, after all) combined with the ease of construction have led to cairns being a common sight throughout the world: they have been found in locations ranging from arctic Scandinavia to desert Africa, from the mountains of Mongolia through to the islands of Hawaii.

## The cairns of the north

Cairns are particularly prevalent in the arctic North America, a region which covers Alaska, Canada and Greenland, to the point where an *inuksuk* features on the flag of the far-north Canadian territory of Nunavut. These structures played a number of roles, but could usually be divided into those used for orientation, hunting, storage, and remembrance.

Possibly the most common role played by cairns is that of orientation. In an environment where there are very few trees, bushes, or other landmarks, a rock cairn acts as an unmistakable marker – someone was here. Multiple cairns may point out a path stretching across the wilderness, a single cairn may show the location of a shoreline to help with seaborne navigation. Even today, cairns are often used as trail markers in mountainous regions or above the tree-line – in high-Alpine environments as in Arctic Tundra, these constructs stand out against the overwhelmingly ‘flat’ background. Cairns were also often used in hunting.

A hunting cairn could be a simple semi-circular blind, behind which hunters could lie in wait for their chosen prey. It could also be part of a complex drive line, essentially a cairn which would look out-of-place to prey in a panic, helping to herd them in the direction of the hunters’ choosing. The meat obtained would then need to be stored and protected from any local scavengers, which is where the storage cairn comes into play. Constructed from local materials and large enough to attract attention when trying to find the food again, storage cairns are well-documented constructs across the Arctic.

Rock cairns are well-known throughout the coastal regions of Canada and Alaska, having been constructed by the native inhabitants for all of the purposes described above. But historically, Native Americans in Southeast Alaska had lived along the coast gathering seafoods. Further, the rugged inland landscape had focused modern development along the coast. Together, history and development concentrated archaeologists’ attention on the coastline – leading to many more discoveries of many



# ‘The oldest cairn was there as Attila the Hun rampaged across Europe, the newest cairn was built when Da Vinci painted the Mona Lisa’



Marianne Okal mapping the Cross Peak survey area with TLS equipment

more sites of interest and thus making a self-perpetuating cycle of discovery. But there is more to the arctic than the coastline and the inland regions were also heavily utilised by native inhabitants – are cairns a feature of the inland alpine regions as well? And if so, why were they made?

## Signs of our forefathers

The story here starts with two elders of the Sitka Tlingit. The Tlingit have been a part of south-eastern Alaska for over 4000 years – living, hunting, loving, dying in an area which requires a keen eye for survival, ever ready to punish the unwary and unprepared. The annual cycle of activities needed to provide food and shelter act as a foundation of their

customs and rituals, in turn providing the common culture holding the group together. The Tlingit call the rock cairns té xóow and generally consider them constructs made by their ancestors in the distant past.

The two elders had heard stories in their youth of cairns high on the peak of a mountain near their family's summer fishing camp. Oral histories referred to them as representing monuments of the Great Flood, built by those who had survived the deluge, but they remained a mysterious presence. To determine the truth of the matter, they turned to a local National Park Service Superintendent and honorary tribal member for help.

Enter Professor William Hunt, who at the time was working as an Archaeologist at the National Park Service and who had recently concluded a 4-year study of Sitka archaeological sites. This background made him the perfect candidate to follow up on the request, and so he was quickly roped into the initial survey. As he recalls, the US Coast Guard flew ‘myself and three others (another archaeologist, an armed Ranger to protect us from brown bears, and historian to represent the park) to two different locations to get more information... the team was amazed to find not just one cairn at each location but nine cairns on one mountain ridge and twelve at the other.’

Why was this so amazing, we might ask? Until this point the common academic belief was that alpine cairns were rare and relatively unimportant – a belief supported by the lack of cairn site discoveries in alpine area. Suddenly, the common consensus seemed to be wrong. Alpine sites were all over the island, the academic field seemed to be ripe for a breakthrough. Hunt was infected with this excitement, ‘I couldn’t stop thinking about them. What were these things? Why were they built? Who built them and when? Why did they occur on the steep mountain sides and ridges above the tree line but within sight of the ocean?’

## Exit right, pursued by a bear

To attempt to answer these questions, Professor William Hunt brought Professor Ralph Hartley into the picture. The two of them successfully obtained funding from the National Science Foundation to follow-up on these preliminary results. Up to this point, the existence of alpine cairns in the area was still quite the mystery – to scientists, to native inhabitants, to everyone. The goal of the project was to brush away some of this mystery, to systematically identify and investigate these cairns so as to find out who made them and when.

Sounds simple, doesn’t it? Well...no. First they had to deal with the location. Baranof Island lies within the Alexander Archipelago, a breathtakingly beautiful region which is perhaps best described a series of impenetrable, rugged mountains rising from the water. The zone receives so much rain that it is officially classed as a rainforest. Grizzly bears stalk the islands, hunting Sitka deer and unfortunate academics. Movement around the inland regions requires a



Ralph Hartley (left foreground), Michael Chodoronek (left background) and Bruce McCune (right) recording a cairn in the Cross Peak investigation area

helicopter, or long days of slow and difficult hiking.

Despite these difficulties the group was able set up camp on a mountain top and began to investigate the region. Their initial steps used LIDAR to survey and image the surrounding terrain down to an exceptional level of detail (LIDAR is essentially a laser which measures the distance from the emitter to the point the beam lands on – repeat this from enough locations and you have a 3D map of the area, mountains and cairns included). This treeless location was covered by ‘pedestrian survey’, an innocuous term for archaeologists’ exhausting hikes throughout the region, eyes always on the lookout for rock cairns or other historical indications. Within a few weeks, an astounding fifty cairns were discovered within about three-quarters of a square mile (about 2 square km). This on-the-ground survey was complemented a year later with a helicopter flights, thought of as a way to quickly locate potential cairns which could be properly assessed afterwards. It turned out to be an exceptionally useful technique, a mere three hours’ survey flight picking out almost 40 cairns across just under 30 sites.

## Leaving no stone unturned

Following the initial survey, four large cairns were picked out for a detailed investigation. The archaeologists’ goals were to recover any artefacts which could assist in determining who built the cairns, and why, as well as to pull out any organic material which might help in detecting when the cairns

were built. Though techniques varied, this process can be thought of as the *extremely careful* removal of each rock, piece by piece, searching through the entire cairn for information, before rebuilding the cairn exactly as it was. As you would expect, this process was only performed after consulting with the Sitka tribe, who consider these sites to be a sacred part of their history.

One of the major questions at the start of the study was a simple: when? When were these cairns made? Despite a complete lack of written history or local artefacts, this question turns out to be answerable through the power of radiocarbon dating and the careful study of lichens growing on the cairns. For those unfamiliar with the technique, radiocarbon dating essentially involves the very careful measurement of carbon isotope levels to determine how long it has been since an organic object (e.g. shoes) was alive (e.g. as a cow). The technique has a certain degree of variability the accuracy tending to be lesser with objects from the more distant past. In this case, the lack of organic objects in the cairns forced the archaeologists to date the soils beneath the cairns. The resulting dates provided a ‘not earlier than’ date for when each tested cairn was built. Discovering the minimum age of a cairn or other surface through the study of lichens growing on a cairn depends on estimating the growth of certain lichens that grow radially – as a circle. A younger lichen patch will be smaller in diameter than a patch that is older. Lichenologists also used a new method of relatively dating cairns through

observing and measuring ‘colonizing’ lichen and plant growths that succeed one another on a rock or on the cairn as a whole. ‘Relative dating’ tells us the sequence of cairn construction but does not provide a date for each cairn’s construction.

Their results? The earliest cairn was built sometime between AD 400–500; the latest between AD 1450–1600. To put this into context, the oldest cairn was there as Attila the Hun rampaged across Europe, the newest cairn was built when Da Vinci painted the Mona Lisa. The amount of history which has occurred between the constructions of these two cairns is the subject of a lifetime’s study, and yet there are many more still waiting in the coastal mountains of Baranof Island. How much more can be learned from these other cairns, mute observers of the Tlingit struggle to survive in the cold northern environment?

We asked Professor Hunt what the next steps in their research would be. ‘As far as future fieldwork and research goes,’ he commented, ‘we have considered doing drone or helicopter inventories of mountains, both inland and coastal, to determine the distributions of alpine cairns across the landscapes of Southeast Alaska. We have also put some thought into looking at similar regions in the world to see if cairns would occur in the same kinds of alpine environments – areas such as southern Chile and Argentina or Norway.’ The difficulty of traversing the inland regions practically requires an aerial survey, and only with a solid grasp of the numbers of rock cairns present can the researchers determine where to focus their sadly limited time.

Both William Hunt and Ralph Hartley are currently Adjunct Professors in the Dept. of Anthropology at the University of Nebraska-Lincoln, roles which have brought them full circle back to their original alma mater (albeit quite a few years later). Not content with turning conventional beliefs on Alaskan cairns on their head, the two are happily planning out a series of future studies. Whatever secrets lie in the frozen tundra, one feels they won’t stay hidden for long.





## Meet the researchers

### Professor William Hunt

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Adjunct Professor William Hunt began his career almost 50 years ago at the University of Nebraska-Lincoln, acting as a Research Archaeologist for the Department of Anthropology just as Professor Hartley was beginning his. With dual M.A.s in Anthropology and Historical Archaeology, his varied career has seen him working as an archaeologist with the National Park Service at its Midwest Archeological Center, a course which naturally brought him to his present role as researcher and educator at the Department of Anthropology.

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#### FUNDING



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Adjunct Professor Ralph Hartley has been in the field of anthropology for forty years, beginning his career as an assistant for the Department of Anthropology at the University of Nebraska-Lincoln. With degrees in history, anthropology and geography, he has covered fields ranging from prehistoric rock art to modern atrocities. Having worked for the National Park Service and the United Nations, his career has come full circle, returning him to the University of Nebraska-Lincoln for his current role.

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## FEEDING THE GLOBAL POPULATION

The first anatomically modern humans – homo sapiens sapiens, the subspecies of homo sapiens that we all belong to – first emerged on Earth almost 200,000 years ago, in the Middle Paleolithic era. Around 190,000 years later, in about 8,000 BC, the entire global human population had grown to be approximately 5 million people. With the rise of agriculture, this figure had increased to somewhere between 200 and 600 million by 1 AD, and finally reached about 1,000 million – or 1 billion – by 1800.

Then an immense change happened with the industrial revolution. Although it had taken almost 200,000 years of human history for the population to reach 1 billion people, it suddenly rose to 2 billion by 1930, 3 billion by 1959, 4 billion by 1974, 5 billion by 1987 and 6 billion in 1999. According to the United Nations, the number of humans inhabiting the planet surpassed 7 billion in October of 2011 – double what it was when John Lennon released his best-selling hit Imagine.

With the population predicted to hit 8 billion in just a few years, and agriculture becoming increasingly difficult due to our changing climate, we are sprinting towards a major global food shortage. So how can we tackle such a global catastrophe? One way is by

producing crops that are robust in the face of climate change, whilst also environmentally friendly and sustainable.

Addressing this challenge head on is the National Institute of Food and Agriculture (NIFA) – the extramural science agency of the United States Department of Agriculture. NIFA provides funding through grants to address food security, in the context of the diminishing land and water resources, climate change, extreme weather events and droughts, and environmental degradation. In the first article of this section of the magazine, we speak to Dr Sonny Ramaswamy, the director of NIFA, who tells us all about the organisation, and its activities in working towards a future of sustainable agriculture.

As rising carbon dioxide levels and increasing global temperatures can create more favourable environments for crop pathogens, one way to ensure nutritional security is by making our crops resistant to disease. In the next article of this section, we introduce the work of Professor Joyce van Eck, who works at the Boyce Thompson Institute – an independent affiliate of Cornell University. Professor van Eck and her team explore new ways to improve the ability of tomato

and potato crops to resist disease using a method known as CRISPR/Cas9. This powerful tool can rapidly perform massive gene alterations, and delete and insert new genes with the help of the Cas9 enzyme. In addition, the team is working on finding new approaches to enhance the nutritional quality of food crops, such as increasing their vitamin and mineral content.

Also working in the field of crop disease-resistance is Dr Corina Vlot-Schuster of the Helmholtz Centre Munich. In the third article of this section we showcase her team's investigations into a natural plant defence system is known as Systemic Acquired Resistance, or SAR. The plant-derived defence molecules involved in SAR may be useful in improving crops without their own SAR systems, and might someday be used as an alternative to toxic pesticides.

To close this issue of Scientia we introduce Professor Daniel Szymanski at Purdue University, also devising new ways to better our crops. Professor Szymanski's research team investigate how protein-based molecular machines give rise to cells of particular shapes and sizes, and explore how this can affect macroscopic traits in the leaves of plants.



# THE U.S. DEPARTMENT OF AGRICULTURE'S NATIONAL INSTITUTE OF FOOD AND AGRICULTURE

An exclusive interview with Dr Sonny Ramaswamy, the director of the National Institute of Food and Agriculture (NIFA)



As one of 18 agencies within the United States Department of Agriculture (USDA), [the National Institute of Food and Agriculture](#) (NIFA) is the extramural science agency. NIFA provides funding through grants to catalyse transformative discoveries, education, and engagement to solve societal agricultural challenges. The agency's mandate is to address nutritional security, in the context of the diminishing land and water resources, climate change, extreme weather events and droughts, and environmental degradation, and ensure the well-being of families and communities.

NIFA partners with America's [Land-Grant University \(LGU\) System](#) of major public universities, which have a presence in every U.S. state, county (district), and territory. The LGUs, which have the tripartite mission of research, extension, and teaching, include also [historically black colleges](#), [tribal colleges](#), [Hispanic-serving institutions](#), and [Alaska Native-Serving and Native Hawaiian-Serving Institutions](#).

NIFA's unique quality is that its investments, which result in cutting-edge discoveries, are translated into innovations and solutions by applied research and extension personnel and delivered to the end users to put knowledge into practice. Here, we speak to Dr Sonny Ramaswamy, the director of NIFA, who tells us all about the organisation, and its activities in working towards a future of sustainable agriculture.

**You have had a very varied and successful career in the agricultural sciences, including undertaking research that was NIFA funded. What motivated you to pursue a career within the organisation?**

I have been in academia all my life – it's as though I never left college – and have been a successful scientist, educator, and administrator at several Land-Grant Universities in the United States. I became the dean of the College of Agricultural Sciences at Oregon State University in 2009 during the depth of the global economic recession, and the budget situation was incredibly difficult. We were able to work with the stakeholders, legislators, and donors to successfully mitigate the impact of the intense budget cuts and continued to ensure the college offered the best education to students and undertook the best research and extension in support of the varied and vibrant agricultural and food scene in Oregon.

We had just started to make significant progress when, out of the blue, I received a telephone call on the 8th of August 2011, which changed my life: the caller informed me of [President Barack Obama's intent](#) to appoint me as the director of the National Institute of Food and Agriculture. I remember I was driving that day to the airport in Portland, Oregon for a fundraising trip to the US Midwest; my knees started shaking and I needed to pull over to complete the conversation. I was stoked and shared the news with my wife and daughter; there was no doubt that I would step up to accept the appointment – how could anyone, particularly an immigrant, refuse to serve one's nation!

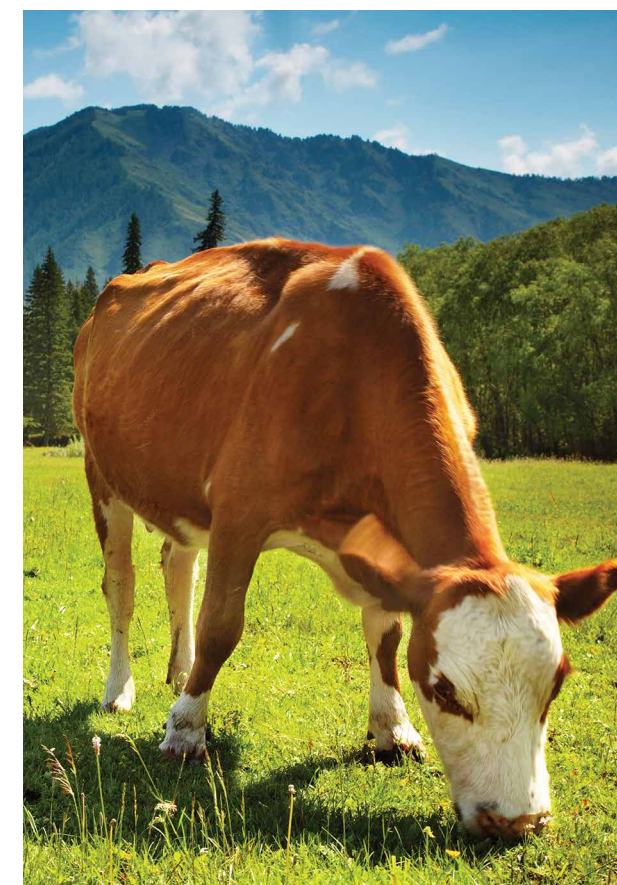
With much regret we left Oregon State University, an excellent institution, and Oregon, one of the most beautiful places in America.

Following nine months of an intense security clearance process, on 7 May 2012, I was sworn in to office by US Department of Agriculture Secretary Tom Vilsack. This was not a position I sought; however, I have had significant success and have enjoyed serving as director of NIFA.

**What is NIFA's annual research budget and what types of grants are available from NIFA?**

For fiscal year 2016 (Oct. 1, 2015 - Sept. 30, 2016), NIFA has a total budget of nearly US \$1.5 billion. NIFA supports research, education, and extension efforts primarily through two types of funding opportunities known as Capacity Grants and Competitive Grants. Capacity Grants ensure that the Land-Grant University (LGU) System and other partners maintain the 'capacity' to conduct research and extension activities. The amount of funds provided to each institution is determined by a statutorily defined formula that may include variables such as the rural population, farm population, number of farms, forest acreage, and poverty rates.

NIFA awards Competitive Grants for fundamental and applied research, extension, and higher education activities, as well as for projects that integrate research, education, and extension functions. America's flagship competitive grants program, the Agriculture and Food Research Initiative (AFRI), will provide \$350 million in Fiscal Year 2016. Competitive programs enable NIFA to attract a large pool of applicants to work on agricultural issues of national interest, and to select the highest quality proposals submitted by eligible individuals, institutions, or organisations. Awards are made following a rigorous peer-review process. Individuals, institutions, or organisations may apply according to criteria listed in each [Request for Application](#).



Typically, eligibility for the Capacity Programs funding is restricted to the Land-Grant Universities or other specified institutions. Competitive program eligibility is broader, and may include Land-Grant and non-Land-Grant public academic and private academic institutions, government agencies, non-governmental organisations, the private sector, community based organisations, and individuals. US-based grantees may partner with international collaborators.

**Please tell our readers a bit about the ways in which NIFA works to sustain natural resources and the environment. On this same thread, explain the means by which NIFA is working to mitigate the impacts of climate change?**

One of the [Farm Bill priorities](#) is Bioenergy, Natural Resources, and Environment, which provides NIFA the mandate to invest in natural resources and the environment, including climate change and water. NIFA takes a holistic approach to maximising food production that is environmentally, economically, and socially sustainable. We recognise that there is no 'one-size-fits-all' solution and consider a diversity of needs for different crops, livestock, and the communities that farm and consume them.

For example, through the AFRI program, NIFA funds several Coordinated Agricultural Projects (CAPs). Research teams funded by CAPs use research, education, and extension approaches to study and implement agricultural practices that sustain natural resources and the environment and work to mitigate the impacts of climate change. CAPs are large-scale, multi-million dollar projects to promote collaboration, open communication, and the exchange of information while reducing duplication of effort and increasing coordination among individuals, institutions, states, and regions. One good example of one of NIFA's currently-funded CAPs is the [Regional Approaches to Climate Change](#).



([REACCH](#)) project, led by the University of Idaho, which aims to enhance sustainability of Pacific Northwest Agriculture (PNW) cereal systems and contribute to climate change mitigation. REACCH implements sustainable practices for cereal production, reduces greenhouse gas emissions from agriculture, collaborates with stakeholders and policy makers, and prepares professionals to address climate change in agriculture. Another interesting project led by Washington State University, called the [Northwest Advanced Renewables Alliance](#), is developing the chemistry and infrastructure for converting forest residuals (twigs and small limbs leftover after timber harvest in slash piles) into bio-jet fuel. An Alaska Airlines demonstration flight using fuel developed by NARA and partners is planned by 2017.

NIFA also funds a number of additional projects on adapting agriculture to drought, flooding, and water conservation. One example is the [AFRI Climate Variability and Change Challenge Area](#), which supports research on beef cattle selection and management for adaptation to drought.

#### How does NIFA facilitate international collaboration?

NIFA participates in several international collaborations to mitigate and prevent the spread of crop and livestock disease, including the United Kingdom's Biotechnology and Biological Sciences Research Council (BBSRC), the US-Israel Binational Agricultural Research and Development (BARD) program, and with the Republic of Ireland Department of Agriculture, Food, and the Marine (DAFM) and the Northern Ireland Department of Agriculture and Rural Development (DARD). In 2014, NIFA and BBSRC collaborated in a pilot program to receive and review US-UK collaborative proposals in animal health. The program resulted in five jointly funded research awards totalling more than \$2.3 million from NIFA and \$3.5 million from BBSRC. Additionally, the Ecology and Evolution of Infectious Diseases Program, a partnership between the US National Science Foundation, the National Institute of Health, NIFA, and BBSRC annually funds projects that address crop and farm animal disease ecology. Finally, the [International Wheat Yield Partnership](#) is a group of funders that collaborate to invest in projects aimed at increasing the primary yield of wheat.

## 'I received a telephone call on the 8th of August 2011, which changed my life: the caller informed me of President Barack Obama's intent to appoint me as the director of the National Institute of Food and Agriculture'



#### How does NIFA approach the challenge of managing trade-offs between maintaining forestry and generating farmland to meet ever-increasing food demands?

The trade-off between producing food and having forests and their products and services is not the real question here. The big picture question is: How do we as a nation – as well as a global community – allocate a finite resource – land – to the various uses that society relies on, such as for the production of food and wood fibre, carbon sequestration, biodiversity, and for housing and commercial development.

NIFA's approach to meeting the food requirements of the ever increasing human population is from the perspective of facilitating research on enhanced productivity of crops and livestock, increasing water, nitrogen, and phosphorus use efficiency, enhancing drought tolerance and pest resistance, and last, but not least, promoting sustainable consumption and mitigating food waste and food loss. As a result, our expectation is that we will not need to bring in additional land into use for production purposes and can, indeed, also mitigate destruction of forests.

What our nation needs is a vision, supported by policies and economic incentives, that promotes making wise decisions about how we use the land for all of its necessary uses. NIFA's work is not about making decisions about how land resources are allocated – our work is to advance the science, which includes research, extension, and higher education, by supporting our university partners as they seek to expand the knowledge and practice of efficiently growing healthy food on the agricultural lands we have and to do so in ways that provide food for consumption, income for farmers and ranchers, and economic resiliency for rural communities, while supporting the science of sustaining healthy and productive forests on the land base that is available for forests.

NIFA has supported a \$20 million project, [PINEMAP](#), to study loblolly pine across its entire range in the southern U.S. and determine how this important forest can continue to be productive in the face of a changing climate. This work has involved studying how various genetic strains of loblolly pine react to changes in precipitation, one of the primary climate change impacts in the southeastern US, while at the same time being more productive



with regard to wood products. Further, the new knowledge is being transferred to forest landowners who will make decisions on what loblolly seedlings to plant based on their genetics and how to manage loblolly pine forests to adapt to and mitigate the changing climate.

#### Please tell us about some interesting and imaginative projects that NIFA is involved with in working towards a future of sustainable food.

The bulk of NIFA's investments support traditional crop and livestock agriculture, nutrition, food safety, forestry, and aquaculture. NIFA also funds some 'out of the box' disruptive projects that work toward a future of sustainable, environmentally friendly food sources, including the development and deployment of smart systems, sensors, drones, imaging systems, and robots. These projects, in combination with knowledge derived from investments in molecular biology, phenomics and phenotyping, and big data, will promote food production with significantly reduced ecological footprint, while ensuring profitability and economic well-being.

For example, NIFA has invested in the development of robotics that facilitate precision agriculture and aid the harvesting process. One such project involved a team of several institutions led by Carnegie Mellon University and which created a camera-equipped vehicle with the ability to detect fruit and conduct an automated image analysis. The image data is converted into measurements of yield components, such as fruit count, size, and quality. In another related project, researchers at UC Davis developed 'co-bots' that are inexpensive, relatively small, and aid humans with the harvesting of fragile crops by supplying them with empty containers and by transporting containers filled with harvested crops to unloading stations. These robots reduce harvesting

time by transporting hand-picked crops. The robots also protect worker health by reducing slipping accidents. [The prototype](#) navigates in furrows and regulates its speed to reduce vibration.

#### NIFA serves as a vital contributor to science policy decision-making in the US. Please tell us about some of your success stories to date in this regard.

NIFA funds several projects that influence policy decision-making in the United States. For example, the Environmental Protection Agency used results from [the Center for Agricultural and Rural Development's](#) (CARD) biofuel model in deciding to temporarily waive the conventional biofuel mandate because of the 2012-2013 drought. The mandate typically requires a certain portion of the American motor fuel supply to include biobased ethanol. Drought conditions, however, caused corn production to decline and prices to increase. Therefore, without a waiving the mandate to produce corn-based ethanol, corn prices would rise even more dramatically, resulting in increased prices of animal feed and potentially hurting consumers. CARD's biofuel model helped show that concerns over the potential negative impacts of temporarily waiving the mandate were not warranted. The team also presented a summary of the water quality effects of corn-based biofuels relative to cellulosic feedstocks to the Roundtable on Environmental Health, a bi-annual policy conference supported by the National Institute of Health and attended by senior staff from major leading federal agencies and policy advocates in Washington D.C.

Similarly, the [National Agricultural and Rural Development Policy Center \(NARDeP\)](#) provides timely and cutting-edge research on current and emerging public policy priorities and regulations in a quantitative format. This initiative creates and disseminates new datasets from



secondary and other sources to policymakers and other interested individuals, serves as a clearinghouse for technology diffusion and educational resources and to disseminate impartial information through web-based training and other publications, and helps to train the next generation of policy analysts. One NARDeP [case study](#) investigated which kinds of policies are best for stemming the outmigration of farm labour, given the unrelenting pressure to also continually develop productivity by increasing forms of technology which inherently are labour-saving. This was in response to [recent USDA Economic Research Service data](#), which shows that labour use in agriculture declined by 78 percent between 1948 and 2011, even as agricultural output more than doubled, increasing by nearly 250 percent. This study found that investments in [Cooperative Extension](#) have been remarkably effective in keeping farmers in agriculture, over the years 1984–2010. In fact, the authors estimate that 137,700 farmers stayed in farming over this period, who would otherwise have exited had they not benefited from the extension and associated research programs. The study showed that extension spending was directly associated with higher net farm income, which in turn allowed the farmers to continue farming. One of the policy implications of these findings was that public investments in farmers are better made through the research and educational programs of the LGU system, rather than through direct subsidies to farmers, if the policy goal is to keep farmers on the farm.

**Additionally, NIFA appears to play a huge role in public outreach and education, please tell us a bit about your activities.**

NIFA is one of several US government agencies participating in the [Federal Science, Technology, Engineering, and Mathematics \(STEM\) Education 5-year strategic plan](#) to improve STEM education across America. NIFA and other USDA agencies are partnering with the National Science Foundation and Smithsonian Institution to improve graduate fellowships and providing resources and materials for engaging learners with science. NIFA is also able to leverage its partnership with the LGUs, the Cooperative Extension System, and its regional networks and laboratories. We are also able to reach a very diverse set of stakeholders through our numerous types of LGUs located in both rural and urban communities. Through targeted funding programs to reach out to minority and underserved populations, we support



institutions that include Historically Black Colleges and Universities (HBCU), Hispanic-Serving Institutions (HSI), Alaska Native-Serving Institutions, Native Hawaiian-Serving Institutions, Insular areas institutions, and Tribal Colleges and Universities.

NIFA is committed to training the next generation of agricultural scientists. Agriculture is a STEM enterprise, and it is critical to attract more youth from an early age and equip them with the STEM skills required for developing the technologies that will address the emerging issues in agriculture and protecting quality of life. This is a global challenge. In addition to the training opportunities offered through the [AFRI ELI](#) and the education component of the CAP grants, NIFA has several other programs that offer educational opportunities, including the [Multicultural Scholars Program](#), the [Women and Minorities in Science, Technology, Engineering, and Mathematics Fields \(WAMS\) Grants Program](#), [Community Food Projects](#), the [Expanded Food and Nutrition Education Program \(EFNEP\)](#) and the [Beginning Farmer and Rancher Development Program](#).

Finally, the Cooperative Extension System ensures that the science NIFA funds is not solely for the sake of science, but rather is translated into innovations and solutions and delivered to the end-user. Extension provides a clear path of bringing research to

the people and gives NIFA a presence in every one of the 3,143 counties, boroughs, and parishes in America, both rural and urban, rich and poor.

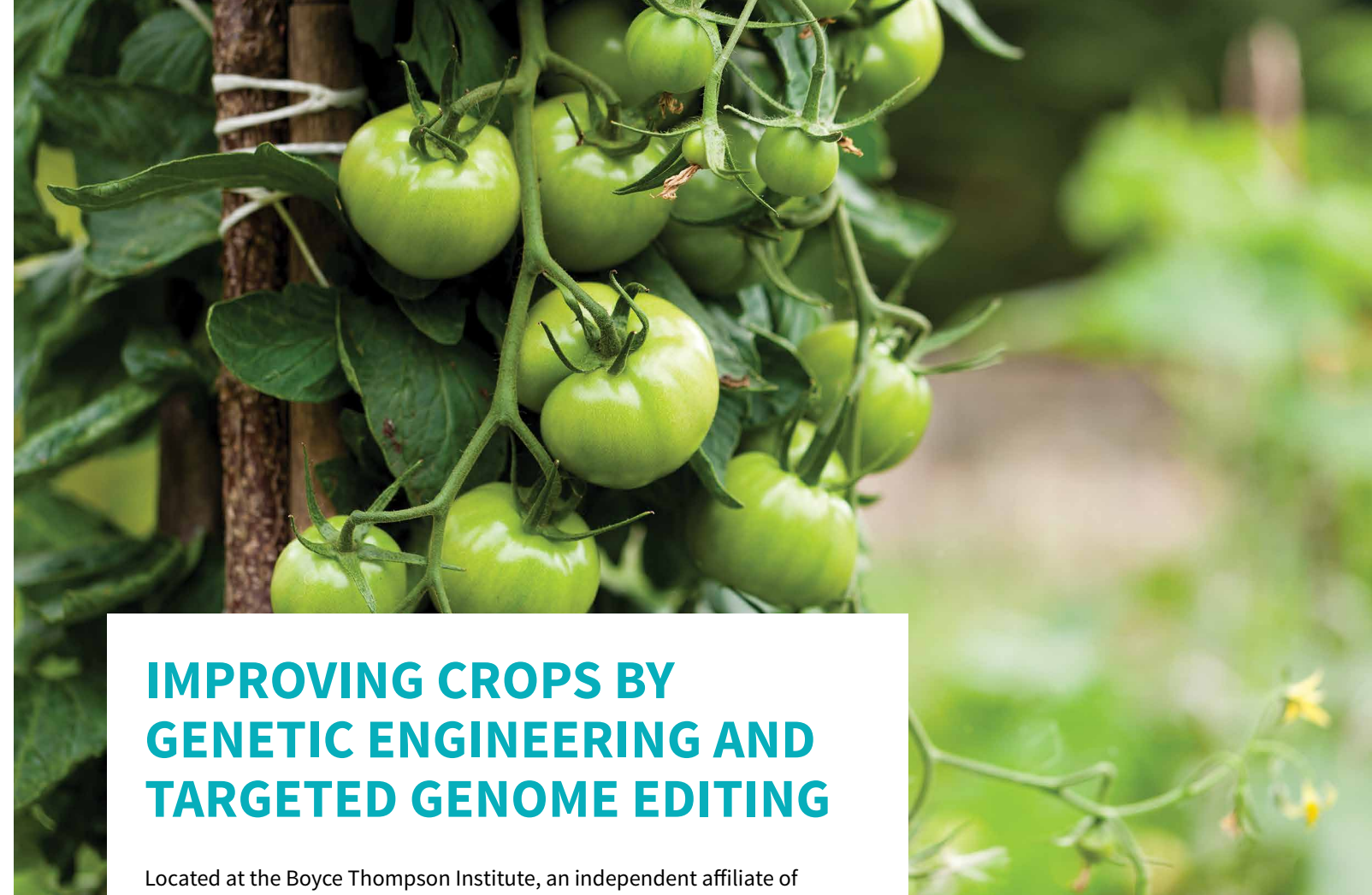
**Finally, what do you see as being the biggest global challenge that NIFA will face in the next 20 years?**

The biggest global challenge that NIFA will face in the next 20 years is to ensure nutritional security, environmental security, and economic security of the burgeoning population, projected to reach almost 450 million in the United States and over 9 billion globally by the year 2050.



United States Department of Agriculture  
National Institute of Food and Agriculture

[www.nifa.usda.gov](http://www.nifa.usda.gov)



## IMPROVING CROPS BY GENETIC ENGINEERING AND TARGETED GENOME EDITING

Located at the Boyce Thompson Institute, an independent affiliate of Cornell University, the Van Eck lab explores novel methods for major crop improvement possibilities offered by a powerful gene editing method called CRISPR/Cas9.

### Portrait of a bioengineer

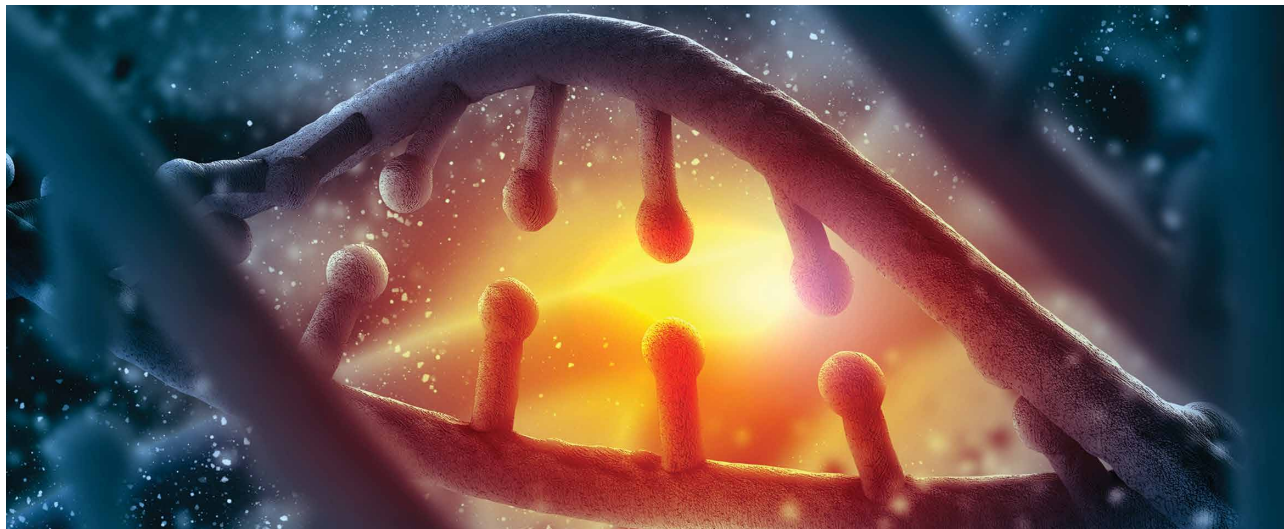
Professor Joyce Van Eck was passionate about science from a very early age. Her interest in science spiked in elementary school, where she especially enjoyed performing experiments. During her secondary education, she was first acquainted with advanced biology and genetics, which she instantly became fascinated by. As she advanced through school, she considered specialising as a paediatrician with a focus on genetic counselling. However, in college she realised that the path to becoming a paediatrician was burdened with dissecting organisms during laboratory classes. To avoid the unpleasantness of this practice, she chose to reorient her studies toward other areas of expertise that combined her passion for genetics with different types of research. It was then that she realised the great potential of plant research, particularly that involving crop improvement and plant breeding. 'I was totally amazed and imagined all the

possibilities for helping to improve crops to resist disease and insects, plus other traits that could be of benefit to crop productivity.' Professor Van Eck explains, 'I was especially intrigued by how genetic engineering could be helpful when traits of interest like disease resistance were not already found in plant germplasm that could be exploited by plant breeders to incorporate into their crop improvement programs.' Many of our crops do not have the necessary genetic traits for resisting disease, and therefore their survival must be aided by genetic engineering, a domain of great interest to Professor Van Eck.

Professor Van Eck went on to receive her PhD for a thesis on the 'Transfer of large amounts of DNA via somatic hybridization and particle bombardment'. Having successfully completed her education and thesis research, Professor Van Eck used her expertise to teach students, advise industrial companies about how to develop new gene transfer methods, and create plant-made vaccines. In 2008, she became the Director



**‘I was totally amazed and imagined all the possibilities for helping to improve crops to resist disease and insects, plus other traits that could be of benefit to crop productivity’**



of the Boyce Thompson Institute Centre for Plant Biotechnology Research, where she is also an Assistant Professor since 2013. The Boyce Thompson Institute is an independent affiliate of Cornell University. She leads a research lab with a focus on biotechnological approaches to the study of gene function and crop improvement. Throughout their research, the Van Eck lab applies several genetic engineering strategies to two major food crops, namely potato and tomato. ‘The focus of my work is to use biotechnological approaches to identify genes of interest that can help improve plants and to study the function of those genes.’ Professor Van Eck explains. ‘My group also develops new genetic engineering systems and improves existing systems to make them more efficient. Through our work we are able to determine the genes that would have the most impact on traits to help improve the productivity of crops.’ Amongst other methods, the team uses CRISPR/Cas9, a powerful genome editing tool inspired from the inner workings of bacteria. CRISPR is a genetic library that can rapidly perform massive gene alterations, and delete and insert new genes with the help of the Cas9 enzyme. In addition, the team is working on finding new approaches to enhance the nutritional quality of food crops, such as increasing the vitamin and mineral content. Aside from their own projects, Professor Van Eck and her team also support various other research groups in their discoveries. ‘We especially enjoy helping groups and knowing we played a role in their discoveries,’ she tells Scientia.

#### Threatened species conservation efforts

One of Professor Van Eck’s projects involved developing a method to help understand the potential extinction of an endangered plant species. This phenomenon often occurs due to new and threatening conditions arriving in the plant habitat, such as climate change, herbivores, and the invasion of competitive plants. *Agrimonia rostellata*, or beaked agrimony, is considered an endangered plant in New York State, as it remains in only 17 locations. The plant is a perennial herb living in central and eastern US, which grows in woods, rocky environments and on stream banks. Being on average about 60 centimeters tall, *Agrimonia rostellata* grows small yellow flowers and fruits. The reasons why it is vanishing are difficult to understand, because research requires taking seeds from the already small pool of remaining plants. To fully understand species decline, these studies need large populations which can be tracked over several years, so scientists must increase the number of plants in the lab if they are to conduct studies to understand and prevent extinction. Professor Van Eck’s lab developed an in-vitro method to do just that. The team worked with 15 plants obtained from three separate locations, from which they took nodes necessary for growing new plants. However, the greatest threat they encountered when propagating *Agrimonia rostellata* crops in the lab was the development of a fungal infection. A major challenge was to find an approach to remove the fungus that did not have a negative effect on plant growth, and worked on the fungal

spores as well. The nodes were kept separate from each other in test tubes and rinsed in several anti-fungal solutions, and were then prepared for plantation once new plants developed from the nodes. This threatened plant species conservation and propagation effort is just one example of the capabilities of plant science. As the climate continues to change and more species become threatened, similar efforts are necessary to protect and recover the diversity of our flora.

#### Testing CRISPR/Cas9 on tomatoes

Ever since it was first reported in 2013, CRISPR/Cas9 has become the tool of choice for geneticists who edit plant genomes. CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats, which are segments of prokaryotic DNA composed of short repetitions of DNA base sequences. These sequences are separated by a piece of spacer DNA, with an isolating role and no encoding properties. Cas9 is an endonuclease – an enzyme that cuts DNA strings into pieces – and has the ability to recognise specific DNA sequences, which helps to guide it to where to make the cuts. When it first appeared, the CRISPR gene editing tool was only used on human and bacterial DNA. Professor Van Eck and her colleagues were the first to test if this technique could be successfully applied to the tomato plant. At the time of the research, the tool had already been used on wheat, rice and sorghum, but it was unknown whether its applicability would extend to any type of plant. Moreover, it was not clear



Photographs courtesy of Sheryl Sinkow

**‘The focus of my work is to use biotechnological approaches to identify genes of interest that can help improve plants and to study the function of those genes’**

whether the genetic modifications would always propagate to the next generation of plants, or if it this was only limited to the stable transgenic lines. In order to test whether CRISPR worked for tomatoes, the researchers chose to delete a portion of a gene whose interrupted function produces wiry, needle-like leaves early in the process of plant growth, such that the mutation is immediately recognisable. The research led to the successful modification of the recovered plants. Most modified plants presented the expected results; however, one of the plants interestingly exhibited genomic deletions in an unintended region upstream from the targeted part of the DNA chain, and another three presented unexpected gene deletions as well. Following the first part of the experiment, the researchers proceeded to test whether the modifications are passed onto new generations. Because they knew the modified plants will have low fertility,

they tested for heritability by using their pollen to fertilise flowers of other wild-type tomato plants. The low fertility only yielded four new plants, but they were all tested and confirmed positive for deletion. Overall, the findings confirmed the excellent potential of using CRISPR/Cas9 in the genome editing of tomatoes. The complete description of the research can be found in a paper on ‘Efficient Gene Editing in Tomato in the First Generation Using the Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-Associated9 System’.

#### Research models and future works

Tomatoes are used very often as models in plant biotechnology research because they are a vastly important crop for the worldwide economy and because of the availability of many resources including a high quality genome sequence and mutant

species. Therefore, Professor Van Eck’s team investigated an approach to improve the genetic engineering methodology and the results are reported in a paper titled ‘Modification of plant regeneration medium decreases the time for recovery of *Solanum lycopersicum* cultivar M82 stable transgenic lines’. A cultivar is a plant variety resulting from cultivation by selective breeding, whereas transgenic lines can be understood as gene implants where researchers use microbes or other methods to transfer genes into the target organism. In this way, tomatoes can gain immunity to viral or bacterial infections. Therefore, it is important to have reliable and efficient methods with a high probability of gene transfer success. The paper studied gene transfer methods for tomatoes and explored whether the transgenic lines can be recovered faster when aided by components of the medium in which the crop was grown. During this research, Professor Van Eck and her team found a growth medium that reduced the time necessary to recover transgenic lines by six weeks in comparison with the regular medium used in such experiments. Moreover, the new medium had no negative results on the cultures. In this experiment, they used *Agrobacterium* to mediate the gene transfer.

When asked about her future research plans, Professor Van Eck explains: ‘The next steps for my research are to develop approaches to elucidate the key steps and genes of the domestication process. In other words, identify the functions of genes responsible for transforming wild species or progenitors into the food crops we know now. A potential outcome of this work is determining biotechnological approaches to developing plant species not commonly used as food crops because of unmanageable growth, small fruits, or low yield and having them become attractive for growers and farmers. In addition, I will continue to exploit genome editing as a tool to study gene function and make targeted changes at gene level to improve plants. We have found CRISPR/Cas9 to be a powerful tool to identify the genes and gene networks involved in plant stem cell proliferation, flowering, and branching in tomato. Tomato serves as a model crop for our work, but our intent is to use what we learn in tomatoes and apply it to other plant species.’





# Meet the researcher

**Professor Joyce Van Eck**  
Assistant Professor  
Boyce Thompson Institute  
Director, BTI Center for Plant Biotechnology Research

Professor Joyce Van Eck is an Assistant Professor at the Boyce Thompson Institute (BTI) and the Director of the BTI Center for Plant Biotechnology Research. She leads a research lab with a focus on biotechnological approaches to the study of gene function and crop improvement. For this research, the Van Eck lab applies several genetic engineering strategies to two major food crops: potatoes and tomatoes. Over the course of her career, Professor Van Eck has been presented with many awards, such as the US Secretary of Agriculture's Honor Award for increasing global food security and Dow AgroSciences' Above and Beyond Award.

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## FUNDING

NSF

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## THE SEEDS WE SOW AND THE GRAIN WE REAP

Much of the world's population is dependent on just a few crops, with blight and disease an ever-present threat. **Dr Corina Vlot-Schuster** of the Helmholtz Centre Munich, after many years researching plant defences against pathogenic attack, aims to open the door to disease-proof crops.

Crops can fail for numerous reasons. Drought can occur, turning the land to windblown dust, or there could be flooding, which sucks oxygen from the soil and turns cropland into rotting bogs. More commonly crops can be attacked by pathogens – plant specific diseases and infections which lead to rotten chunks of fruit, stunted crops, and dead plants.

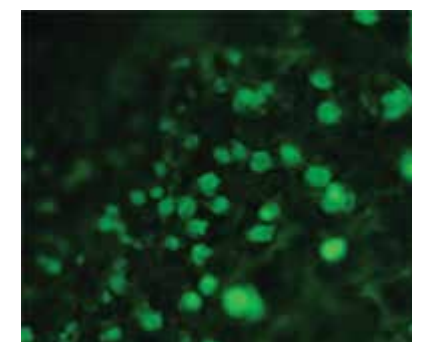
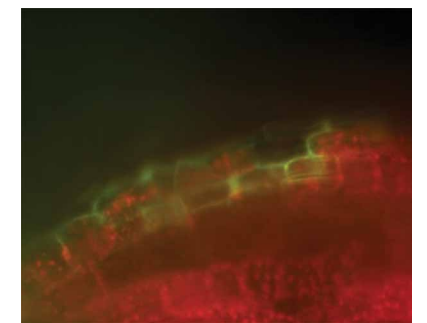
Between 1988 and 1990, failure of our eight major crops (which collectively comprise half of the global croplands) has cost us an estimated 300 billion dollars worldwide. Furthermore, about 65% of crop losses in the U.S. are caused by nonindigenous pathogens, totalling to a cost of about 137 billion dollars every year.

### Molecular thorns

Plants, of course, have been around for a very long time (over 400 million years, in fact), and in that time have often been attacked by disease. Thus, as you would expect, they have developed a number of systems to help

fight off pathogens, and these systems are a promising area of research for scientists trying to develop disease-free crops. One such defensive system is known as Systemic Acquired Resistance, or SAR, and the name itself is a good description of what it does: SAR is systemic because it affects the entire plant, not just the section currently under attack; acquired because it can be activated or induced by the presence of pathogens; and resistance because, well, it heightens plant resistance to attack.

The most important factor here is the 'inducible' part. Unlike many other defensive systems, SAR is only turned on when the plant is actually under attack. SAR activation is triggered by the presence of the 'stress hormone' salicylic acid (which you may recognise as the active metabolite of aspirin), which itself is often associated with the detection of pathogen-associated molecules by specialised receptors in a process which is somewhat similar to our own immune response.





**‘I started working on systemic acquired resistance around 12 years ago and was immediately grasped by the challenging complexity of this biological response, but also by the potential to work towards applied sciences’**



Any defensive system has what is known as an ‘energy cost’, in that energy which is being used to help protect the plant is not being used for getting larger, making seeds, growing delicious fruit, etc. Thus plants which are constantly defending against attack cannot produce the same yield as those which are untouched, and similarly plants which are bred to constant defence will never be as high-yielding as those which are not. Here the advantage of SAR’s inducible nature comes into play, as it has a low energy cost in the ‘waiting’ mode, only rising when it is actually needed. As you would expect, an inducible system of this sort is highly complex, with multiple interacting proteins and molecules controlling the final outcome.

#### Seeing the wood for trees

Attempting to decipher some of this complexity is Dr Vlot-Schuster, of the Institute of Biochemical Plant Pathology at the Helmholtz Centre Munich in Germany. Bringing many years’ experience in plant pathogens and the resultant responses, she has recently been working on unravelling the SAR system in vital crop species such as barley and wheat.

The initial work began in every scientists’ favourite plant, *Arabidopsis thaliana*. A small plant with a fast lifecycle, it is a common model organism for botanical research in the same way that particular mouse breeds are common models for mammalian research. Dr Vlot-Schuster’s early work examined the SAR system in *Arabidopsis*, identifying several proteins which had effects on the activation process. They also utilised metabolic profiling alongside this, essentially a method of fingerprinting a biological state based on chemical entities present (i.e. not just ‘you have high blood-glucose levels’ but ‘based on the presence of these glucose-related metabolites, you have disease X’). The researchers were able to show a distinct ‘SAR signal’, several small compounds which were able to help modulate salicylic acid’s activation of the SAR process – a vital step on the road to controlling the system ourselves.

Practical for inducing SAR in *Arabidopsis*, certainly. *Arabidopsis*, however useful as a model, is not particularly relevant to feeding humans. Dr Vlot-Schuster thus decided to move onto newer pastures, as she commented: ‘SAR was well-characterised in *Arabidopsis thaliana* whereas little was known about SAR in crop plants.

So, around seven years ago, post-doc Dr Sanjukta Dey and technicians Marion Wenig and Claudia Knappe in my lab started transferring experimental methodologies from *Arabidopsis* to barley.’ This was more complicated than it sounds, barley and *Arabidopsis* have different lifecycles, proteins, underlying genetics, even the genome of barley is about 40 times larger than that of *Arabidopsis*.

Their hard work paid off, however, with the identification of SAR-like systems in barley, and it was a lot of work, as Dr Vlot-Schuster says: ‘Currently we are the first and only group to have established a SAR-like pathosystem in this cereal crop’. These are not true SAR systems in the original sense of the word, as they do not appear to be activated by salicylic acid. Instead, the call for help is transmitted by transcription factors, small proteins which act to turn specific genes or sets of genes on and off. Specific activation of genes is a key part of many long-lasting responses to environmental stimuli, the typical example given here is the increased transcription by bacteria of heat-shock-proteins (which help proteins to fold) following a period of higher temperature (which unfolds proteins).

So barley has a SAR-like defence system – why is this important? Dr Vlot-Schuster was happy to comment on the applicability of this research: ‘Plant disease resistance signals can be or become relevant for crop protection. Because SAR is exceptionally resource-efficient (no or very limited yield costs), signals that are involved in this particular disease resistance process are likely to be good candidate molecules to be incorporated in future crop protection schemes using plant-derived defence compounds rather than toxic pesticides that are in use today.’ The discovery of transcription factor elements in the barley SAR response indicates that there are multiple proteins which could be targeted by these candidate ‘plant pharmaceuticals’, significantly improving the chances of success.

#### Peas in a pod

As with many areas of agriculture-related science, plant defence research has to develop in order to join basic studies with on-field application. In this regard, plant phenotyping – basically the identification of unique individuals from a large collection by analysing the most important traits – has

**‘Plant disease resistance signals can be or become relevant for crop protection. Because SAR is exceptionally resource-efficient, signals that are involved in this particular disease resistance process are likely to be good candidate molecules to be incorporated in future crop protection schemes using plant-derived defence compounds rather than toxic pesticides that are in use today.’**



become vital on an international scale. The working group of Dr Vlot-Schuster develops novel tools for analysing plant defence phenotypes with the aim of overcoming the often tedious work of manually screening libraries of plants. Of course, no scientist does their best work alone, and Dr Vlot-Schuster is no exception. She is currently part of the German Plant Phenotyping Network (which goes by the acronym DPPN, for the German ‘Deutsches Pflanzen Phänotypisierung Netzwerk’). This network covers three sites within Germany: The Forschungszentrum Jülich, the Leibniz Institute of Plant Genetics and Crop Plant Research in Gatersleben and her home institute, the Helmholtz Centre Munich. ‘Here in Munich we have several platforms, most prominently the highly acclaimed environmental simulation unit and a recently adapted unique volatile organic compound (VOC) analysis platform as well as the platform in my lab, which is currently called SignalSCREEN. This installation, which will also be accessible for external users, can screen seedlings of different plant species for susceptibility to fluorescently marked pathogens,’ Dr Vlot-Schuster explains.

SignalSCREEN is based on high-throughput confocal microscopy, essentially the ability to use a very high magnification microscope on many thousands of different samples in a very short period of time. High-throughput methods are very popular with pharmaceutical companies, who use them to screen libraries of chemicals for potential new drugs. As each chemical library can contain hundreds of thousands of compounds, high-throughput systems use extensive levels of robotic and software automation, to the point where the machine can often run the entire experiment itself. Confocal microscopy methods are usually paired with fluorescent labelling techniques, in this case for example, plant pathogens are provided with a fluorescent protein (GFP)-tag which allows them to be easily spotted under the microscope – wherever the camera sees brightly coloured spots, that’s another pathogen eating away at the plant. Transferring such a sophisticated method to a high-throughput scale is a novelty and provides us with

the possibility to find resistant individuals in a pool of susceptible plants, thus defining points of action to use natural defence in plant breeding strategies.

Developed in collaboration with Carl Zeiss Microscopy GmbH and Analytik Jena AG, the quantitative approach of SignalSCREEN qualifies it as a state-of-the-art phenotyping platform. It speeds up a specific experimental setup so that no scientist needs to sit in a dark room for weeks to screen a collection of plants. However, it is the emphasis on networking that elevates Dr Vlot-Schuster’s strategy to go beyond basic research and bridge the gap towards field application. Because it is such a new area, it provides the very real possibility that the Helmholtz Centre Munich as part of the DPPN will become a focal point and leader for research in this field – certainly Germany-wide, and perhaps throughout Europe.

#### Budding plans

So where to from here? Dr Vlot-Schuster plans to combine both traditional biochemical methods with the high-throughput options provided by the DPPN network. Her comments are typically modest, aiming ‘to of course continue investigating barley SAR-like immunity at the molecular level. However, we will also apply our phenotyping platform, which can help us further in applied directions.’ The ultimate goal is to be able to control the SAR process in barley, wheat, and other vitally important crops – perhaps using specially developed molecules which would act as ‘plant pharmaceuticals’. By turning the SAR defence system on and off at will, farmers could quickly change their plants between growth and defence mode, leading to better yields and less chance of crop failure. And thus, in the long run, a safer future for all of us.





# Meet the researcher

**Dr Corina Vlot-Schuster**  
Helmholtz Zentrum Muenchen  
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Hailing from the Netherlands, Dr Corina Vlot-Schuster currently works at the internationally renowned Helmholtz Centre Munich, a research centre for Environmental Health that is part of the Helmholtz Association of German Research Centres. Her research career began in the field of mammalian viruses, but she soon changed track to target plant viruses and other pathogens. A successful research career followed, leading to over 20 publications to her name, numerous successful grant applications, and rapid advancement. She is currently a group head at the Helmholtz Centre Munich Institute of Biochemical Plant Pathology, where she leads the Inducible Resistance Signalling laboratory.

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Dr Patrick Schweizer, Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany



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## FUNDING

Work in the Vlot-Schuster lab is currently funded by the Deutsche Forschungsgemeinschaft (DFG) as part of the collaborative research centre SFB924 and by the German federal ministry of education and research (BMBF) as part of DPPN (grant No. 031A053C).



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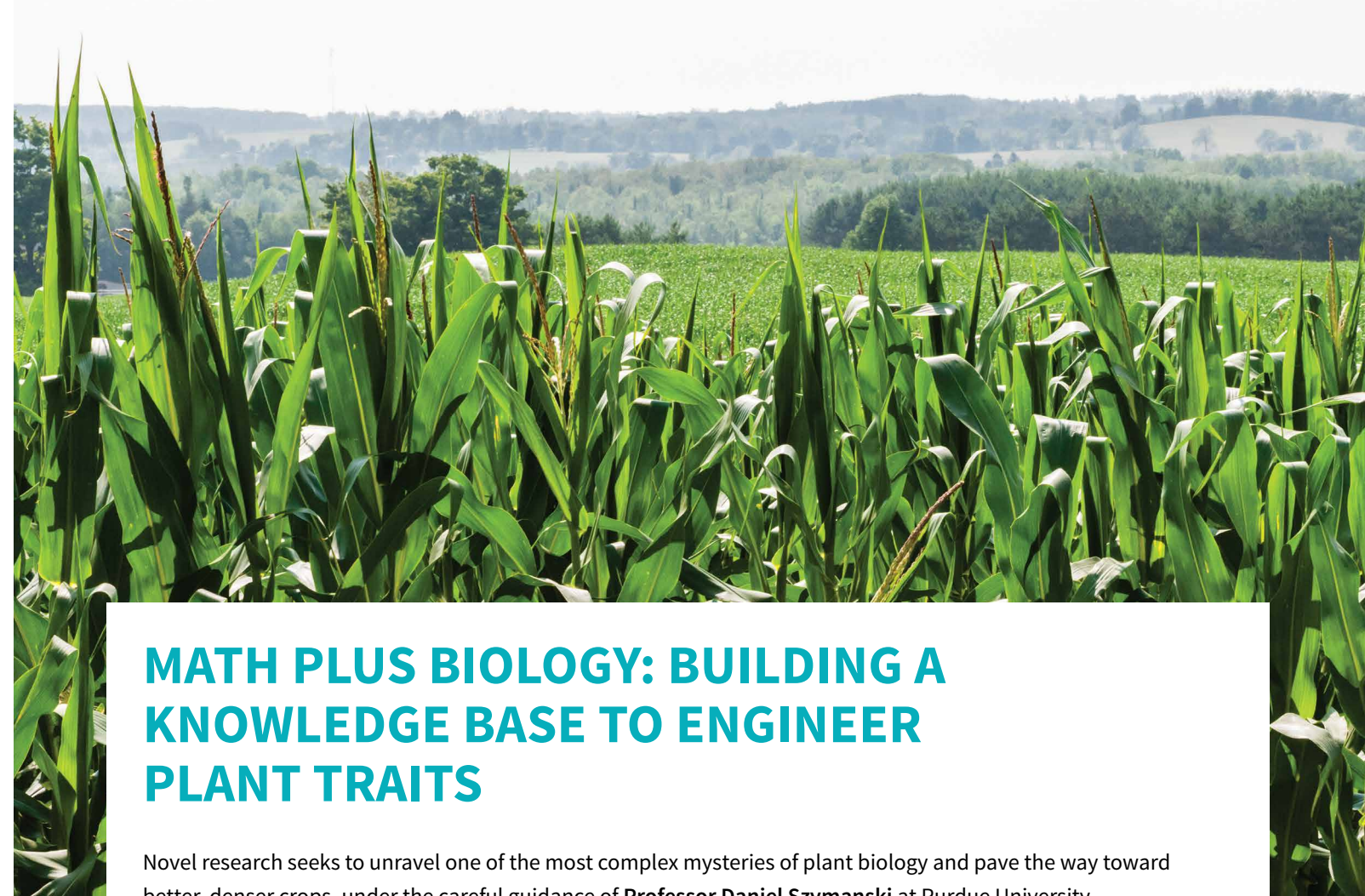
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## MATH PLUS BIOLOGY: BUILDING A KNOWLEDGE BASE TO ENGINEER PLANT TRAITS

Novel research seeks to unravel one of the most complex mysteries of plant biology and pave the way toward better, denser crops, under the careful guidance of **Professor Daniel Szymanski** at Purdue University.

### Looking into Leaf Growth

Trapped within a thick canopy formed by the leaves of their neighbours, plants must compete for limited sunlight that is used to drive photosynthesis. Plants are able to tune their overall architecture to suit environmental conditions, and produce leaves with optimal sizes, shapes and angles. Leaf development is highly complex, as the leaf bud is converted from a cylindrical peg, into a thin, yet mechanically stable, blade-like structure.

Leaf shape is of utmost importance in agriculture. For example, more erect leaf angles have enabled greater planting densities and higher yields per acre for maize. This trait has been achieved through traditional plant breeding and selection. In the future, there will be opportunities to combine breeding and selection with genetic engineering to generate new types of crops with optimised whole plant architectures. Presently this is not possible however, as we lack sufficient knowledge about how genes, cells, and tissues interact across wide spatial scales to control the overall morphology of the leaf. Therefore, there is a strong need

to develop new experimental methods to analyse gene function at the levels of cell, tissue sector, and whole organ.

Professor Szymanski's research team and collaborators investigate how protein-based molecular machines give rise to cells of particular shapes and sizes, and explore how this can affect macroscopic traits. Specifically, Professor Szymanski's area of expertise is the genetics and cell biology of plant growth. His research uses the model plant Arabidopsis for molecular genetics, cell biology, and computational biology studies. In addition, some of his newer projects involve legumes, cotton and maize.

### Discovering the Cellular Basis of Leaf Shape

In the early stages of leaf development, cylindrical leaf primordia are converted into flat surfaces with polarised shapes. The size and shape of the leaf is determined by the number of cells, and the final sizes and shapes of the cells. Plant cells, unlike animal cells, can increase in volume by a factor of 100 or more after they stop dividing.

Therefore, the magnitude and direction of cell expansion can exert a strong influence on the size and shape of the whole leaf. In plants, the outer layer of cells – termed the epidermis – exerts a strong influence over the growth properties of the organ. In many important crop species, the leaf epidermis is comprised of pavement cells that can adopt wildly varying shapes: some of them are polygonal, while others are highly lobed with finger-like protrusions to generate tissue comprised of interdigitated cells with a jigsaw puzzle piece morphology. One major challenge is to determine what controls the complicated shape change in this cell type and to create computational methods to analyse the geometry of shape change over time.

An earlier study carried out by Professor Szymanski and his colleagues focused on defining the developmental progression of how a pavement cell transforms from a simple polyhedral shape to a lobed morphology. The leading idea at the time was that lobe formation was an iterative process determined by cell size, and as the cell grew and the distance between existing lobes increased, new lobes would



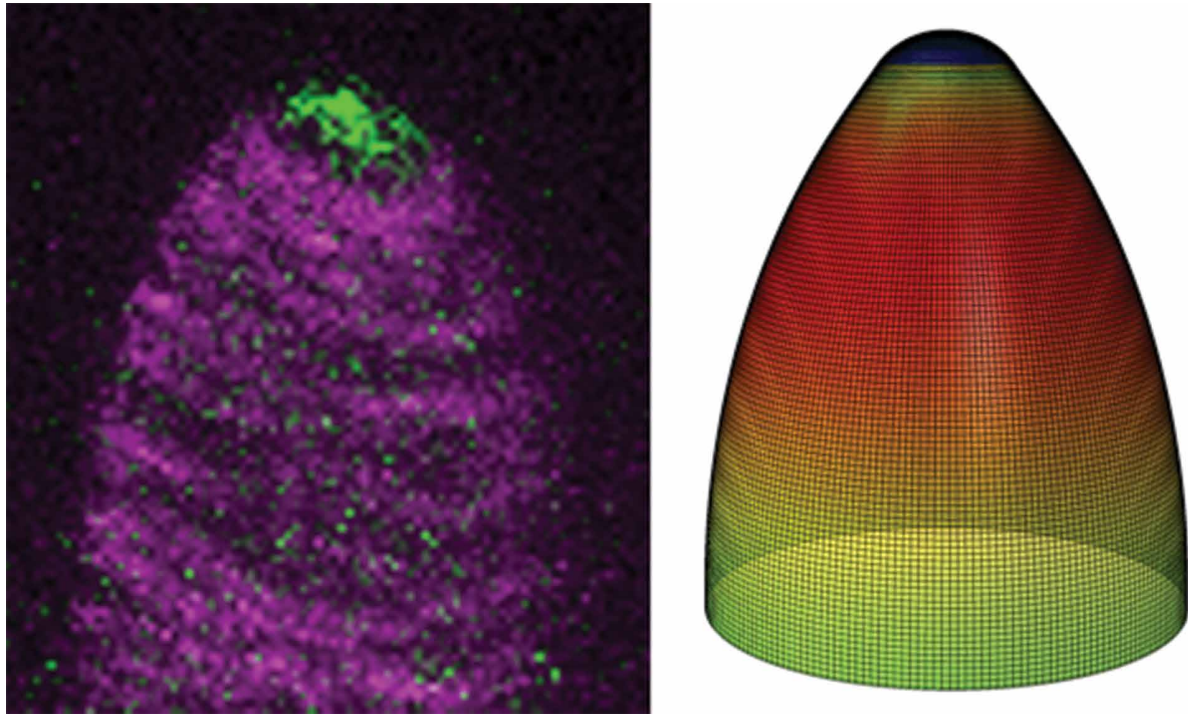


Figure 1. (Left) A live cell image of a real cell with microtubules coloured magenta and the actin filament nucleator ARP2/3 coloured green. (right) A FE computation model of the same cells showing a heat map of the growth patterns of the cell. red (high), blue (low).

form. Through a time-lapse analysis, the Szymanski team found that lobe formation is episodic, and occurs during early cellular development. Once the new lobes have formed, the cell uses this new pattern as a template for future growth that occurs for days. This observation suggests that hormone signalling and other developmental events create a permissive state for polarised growth and these early growth events can have a long-lasting effect on the growth pattern of the cell. The genes and cellular parameters that control this polarised growth process are currently being discovered and analysed.

Another major challenge is to figure out a way to quantify the growth behaviour of cells with such complicated geometric shapes. For a circular or polyhedral cell, it is very simple to graph changes in the size and shape of the cell. However, for pavement cells with an irregular closed geometric shape, how does one measure the formation of a lobe, or measure the growth trajectory of a sub-region of the cell? In a collaboration with the lab of Dr David Umulis – an Associate Professor of Biological Engineering at Purdue – Professor Szymanski's group created a new tool to quantify the formation of lobes and the local geometry of the cell. The computational tool termed LobeFinder (<http://datadryad.org/resource/doi:10.5061/dryad.cs78t>) creates a refined convex hull around extreme points

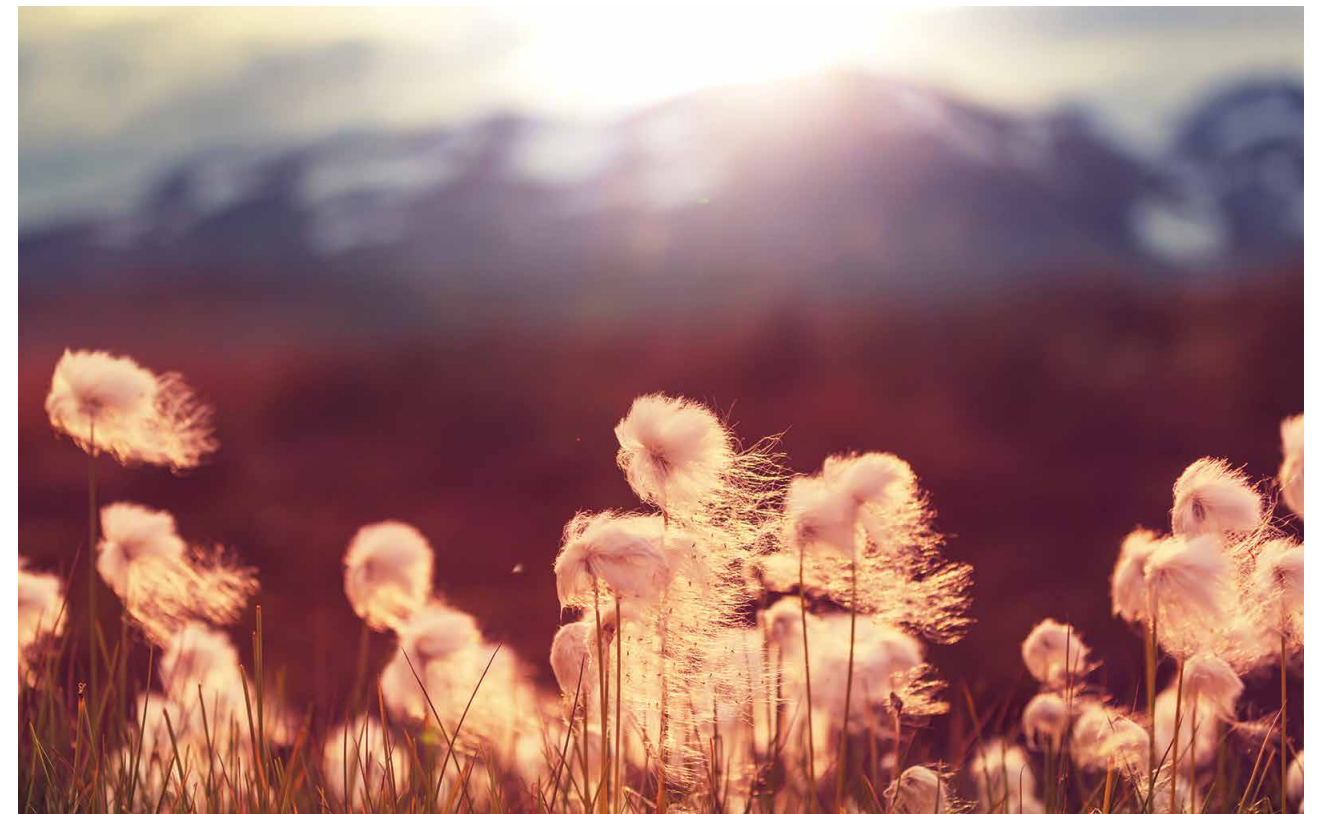
along the cell perimeter, and then measures the distance from the cell perimeter to the convex hull. This method converts the highly irregular shape of a pavement cell into a simple plot of cell shape as a function of location along the cell perimeter. These plots were then used to develop rules to measure lobe formation more consistently than a trained scientist, and much more accurately compared to previous methods. This tool now provides an objective and accurate way to analyse mutant plants to determine if they really do affect the formation of new lobes, or the outgrowth of existing lobes. This is an important first step in generating a reliable genetic pathway that can be used to control cell morphology. The tool also provides a way to graph the timing and location of lobe formation and cell boundary changes. This is important because now proteins that are thought to be involved in the process of lobe formation can be tested directly in microscopy experiments. Do they appear at a cellular location that predicts the location of a new lobe? This tool also allows one to graph how the magnitude and direction of cell growth changes over time. What genes and cellular activities influence these patterns? How do these subcellular and cellular patterns of growth scale to influence the size and shape of a whole leaf? A combined computational and experimental biology approach is creating the knowledge base that is needed for leaf trait engineering.

#### Valuable Single Cells: Leaf Hairs and Cotton Fibres

In many cases an important plant trait is determined by a single cell. For example, individual root hair cells generate most of the surface area of a root and affect the efficiency of water and nutrient uptake. On the leaf surface, hair like cells develop from the epidermis, and they can protect the plant against insect attack. Furthermore, the density, shape, and chemical composition of leaf hairs can have a strong effect on how effectively the leaf is protected from herbivory.

Cotton fibre – the most important textile crop in the world – is comprised of individual hair-like cells that develop on the epidermal cells of the seed coat. The value of the fibre is based on its length, diameter and mechanical strength – all of which are the output of the growth properties of a single cell. Unlike pavement cells, these cell types execute a very regular series of cell shape changes as they morph from a hemispherical cell to a highly elongated cell with a tapered tip. Engineering the mechanical properties of a single cell type is much simpler, and it is now possible to understand how individual genes and their encoded proteins can dictate the growth patterns of an isolated cell.

In a study published by Nature Plants entitled 'Patterning mechanisms of



cytoskeletal and cell wall systems during leaf trichome morphogenesis', Professor Szymanski's research team discovered new ways in which the internal cytoskeleton of the cells influences shape during growth. In plant cells, the shape of the cell and how it changes are dictated by a tough outer cell wall. The inside of the cell is under very high pressure (about 90 psi, similar to a racing bicycle tire) and the manner in which the cell changes shape is dictated by the local mechanical properties of the wall. The softness of the wall, its thickness and the degree to which cellulose fibres are aligned in the cell wall can all influence how the cell changes shape during growth. The primary purpose of the cytoskeleton is to organise the machinery inside the cell that determine the mechanical properties of the external cell wall. In collaboration with Dr Joe Turner, a materials scientist and engineer at the University of Nebraska-Lincoln, the research team created a finite element computational model of the cell wall of a developing leaf trichome in Arabidopsis (see Fig. 1). The finite element method is a computational technique that treats the cell wall like a pressurised shell, and the shell is divided into discrete sectors with specific mechanical properties. The model is used to identify what mechanical properties of the wall are needed to generate a pattern of shape change that is observed within the living cell. A time-lapsed movie of the growing cell provides a way to validate the accuracy of the predictions from the finite element model (click [here](#) for the movie).

The combination of finite element analysis of the cell walls and live cell imaging allowed the team to discover how the microtubule and actin cytoskeletons work together to dictate the shape change patterns of the cell. They analysed the patterning of microtubules and cellulose microfibrils in the cell wall, and found that a specific threshold of fibre alignment transverse to the long axis of the cell must be met in order for the cell to increase in length without any significant radial swelling. The function of actin during plant cell morphogenesis in general is poorly understood, because the actin network is so unstable in most

cell types and its patterns are not easily correlated with cell shape. In this study, the team showed that the evolutionarily conserved actin cytoskeleton nucleator – a protein complex termed the actin-related protein (ARP)2/3 complex – generates a stable patch of actin at the tip of the cell (Fig. 1). This actin patch organises the intracellular roadways for material delivery in the cell. Without the patch, the roadways are random, and new cell wall material is not distributed equally in the growing cell wall. When this happens, the cells become swollen and elongation often stops. The team also showed that the region of the cell apex where the actin patch is present is dynamic, and the size of the patch changes as the cell grows to allow it to become more narrow. This process, referred to as 'tip refinement' allows the leaf trichome to have a sharp tip that defends against insects. In cotton fibres, tip refinement is a very important trait, as the value of elite cotton varieties is derived, in large part, from the smaller diameter of the fibres. New knowledge from the Arabidopsis leaf hair system is being translated to the cotton fibre system.

#### Future Research Directions

Professor Szymanski's work is important because it is generating the knowledge base that is needed to engineer plant cell size and shape. His group has discovered how signalling modules determine the location and timing of actin polymerisation within an individual cell. In the future, these modules can be used in crops such as cotton fibres to engineer fibre quality. The team's combination of experimental and computational biology is also revealing how the actin and microtubule cytoskeletal systems work together in the cell to control the patterns of growth. These computational methods have the potential to be applied to groups of cells within a tissue, in order to direct strategies to engineer the architecture of plant organs or even whole plants. The next step for their work is to develop integrated molecular and mechanical models of cells in the context of a tissue.





## Meet the researcher

**Professor Daniel Szymanski**

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Professor Daniel Szymanski is a Professor of Botany and Plant Pathology, Agronomy and Biological Sciences at Purdue University, USA. He is currently investigating how plant cells use cytoskeletal systems to pattern the cell wall and the shapes of growing cells. He received his PhD in 1995 from the University of Illinois and since then his research has focused on molecular genetics, live cell imaging, proteomics, and computational modelling of plant cell morphogenesis. Throughout his career, he received multiple awards among which are the Purdue Agricultural Research Award and the Purdue Seeds for Success Award. He is a member of the American Association for the Advancement of Science, the American Society of Cell Biology, and the American Society of Plant Biology.

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### FUNDING

NSF (Grant IOS/MCB, Grant No. 1249652 and MCB Grant No. 1121893)  
Purdue Agricultural Research Program  
National Science Foundation Molecular and Cellular Biosciences (MCB)  
National Science Foundation Plant Genome Research Program (PGRP)

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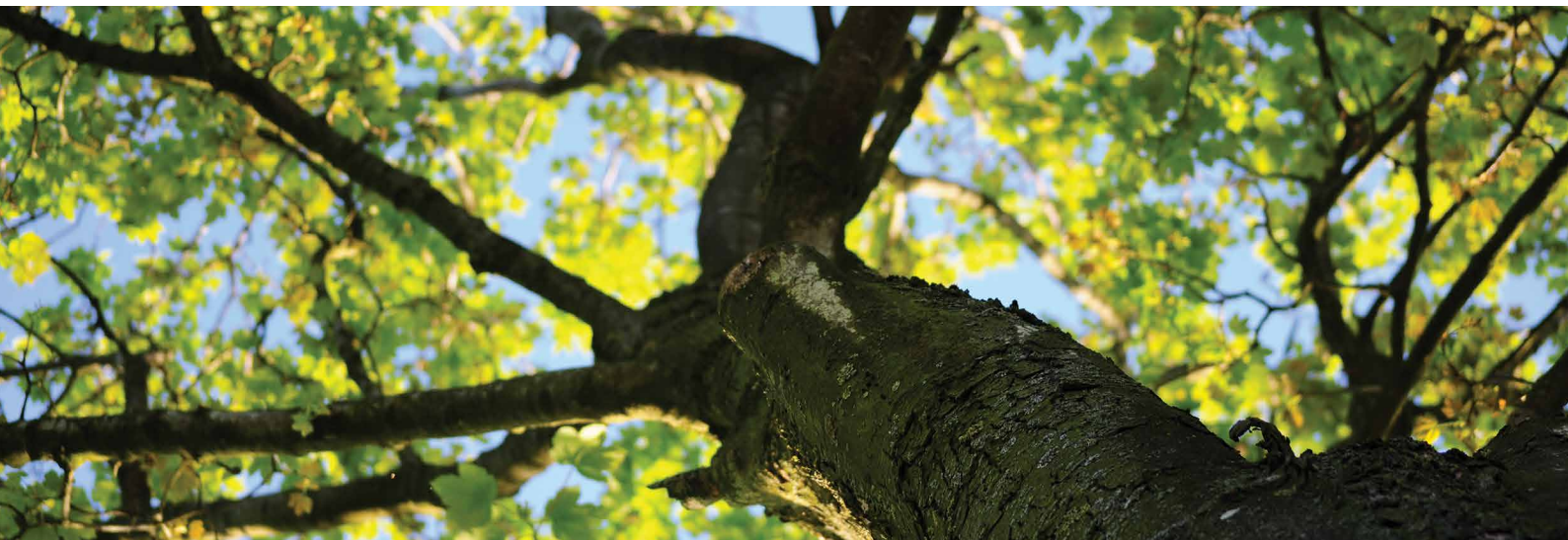


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