

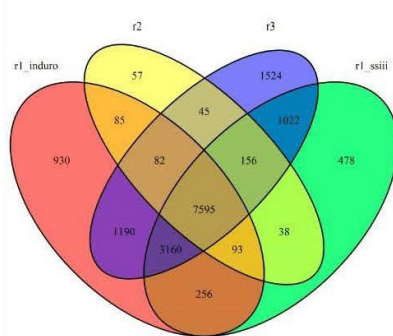


## Analysing myrtle rust's long read transcriptome

**Bhavika Kumar**

Schwessinger Group | Supervisor: Ashley Jones, Ben Schwessinger

*Asutropuccinia psidii* is a fungal plant pathogen invasive on Myrtaceae plant species and commonly called eucalyptus rust, guava rust and myrtle rust. The disease causes deformed leaves, heavy defoliation of branches, reduced fertility, stunted growth, and eventually plant death. The spores of myrtle rust spread easily via clothing, hair, skin, and personal items and first appeared in Australia in 2010 and its rapid spread is impacting the natural vegetation.



Studies have shown that messenger RNA (mRNA) methylation can impact the transcripts stability, splicing, alternative polyadenylation and translation. The aim of my project was to use nanopore direct RNA sequencing to examine the RNA methylation in *A.psidii*. Replica RNA libraries were prepared with induro, a novel reverse transcriptase for nanopore direct RNA sequencing, and sequenced using MinION sequencer. The sequence was basecalled (using guppy basecaller) mapped against the *A.psidii* haplotype 1 genome using minimap2 and 2.5-3 million reads obtained. The CHEUI tool was then used to detect N6-methyladenosine (m6A) and 5-methylcytidine (m5C) RNA modifications using a *Puccinia graminis* reference transcriptome. As summarised in the Venn diagram, of 16,711 sites m6A methylated, 7,595 sites were common in all replicate RNA samples.

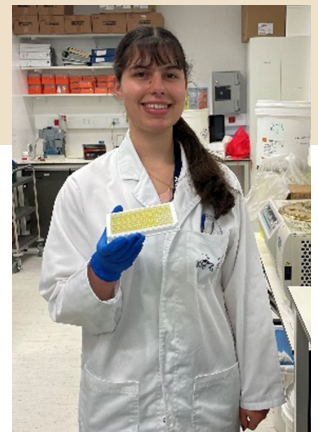
The program has been invaluable for my understanding of bioinformatics and research. The project taught me how to tackle big datasets and most importantly how not be scared when you encounter errors in your

code execution! The experience provided me with the confidence to do research at a higher level.

## Quantifying natural corticosterone variation in skink eggs

**Amelia Peardon**

Noble Group | Supervisors: Daniel Noble



Corticosterone (CORT) is a glucocorticoid analogous to human cortisol, and is found in non-human mammals, birds, amphibians and reptiles. CORT is produced when the hypothalamic-pituitary-adrenal (HPA) axis is stimulated by stress, consequently affecting metabolism and behaviour. In pregnant skinks, elevated CORT causes increased prenatal CORT, affecting offspring development. Studying CORT variation in skink eggs has applications to study maternal effects by determining if the mother's environment and genotype affects the offspring phenotype. Invasive species can be investigated by observing how mothers respond and adapt to novel environments, and the resulting impact on offspring. The aim of my project was to quantify CORT variation between and within egg clutches, and CORT variation between Australian *Lampropholis delicata* (delicate skink) and *Lampropholis guichenoti* (common garden skink) species.



Over the summer, I collected egg clutches from the lizard enclosures, helped prepare new indoor and outdoor enclosures and feed the mass of newly-hatched juveniles. It was so enjoyable working with the lizards and observing how the skinks interacted. The egg dissection proved challenging as they are soft and small (sampled a double-yolk!). I then isolated steroids from the yolk samples using Solid Phase Extraction to remove lipids and proteins and used an Enzyme Immunoassay to measure the CORT levels. I discovered evidence of CORT variation both between and with egg clutches and between skink species, with higher CORT levels produced in *L. delicata* eggs.

I really enjoyed my time as a biology summer intern. I am very grateful for the opportunity to work alongside and be guided by such passionate and caring researchers, as well as the opportunity to hear research from others in the field, and even be featured on the ANU College of Science website! Overall, the Summer Research Internship has been a very rewarding experience and has significantly increased my knowledge and appreciation of research, and gain valuable skills which I will use for the remainder of my studies and beyond.



## Pathogen Resistance in Cereal Crops – Interaction of R and Avr genes

**Elena De La O**

Rathjen Group | Supervisors: Xiaoxiao Zhang, John Rathjen

It is estimated 16% of global crop yields are lost to pathogens. Genetically modified pathogen resistant plants may therefore be an important part of the solution for greater food security. Working collaboratively with Lucy Molloy, we were tasked to investigate the interaction between plant disease resistant (R) and pathogen effector (Avr) genes, aiming to identify novel

disease resistance mechanisms in important crops wheat and barley.

Our project involved cloning R and Avr genes into our expression plasmids using Golden Gate molecular cloning and checking for their successful transformation using restriction enzyme digestion and DNA Sanger sequencing. Nine of 13 genes were successfully cloned and tested to assess whether the recombinant plant R immuno receptors were recognised the Avr pathogen effectors using a split-luciferase assay designed to detect immune response triggered cell death. AvR-R interactions were also tested in whole leaf necrosis assays by transforming our genes into *Agrobacterium* and transiently expressing them in the model protein expression plant *Nicotiana benthamina* by infiltration.

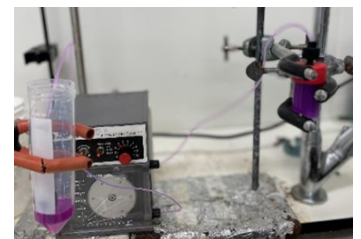
In eight weeks, I have met many great people and gained a greater understanding of what research encompasses. Under the supervision of Xiaoxiao Zhang, I have developed my laboratory and research skillset, and am inspired to seek more research experience during the remainder of my undergrad.

## Using nanobodies to mediate plant immune system interactions

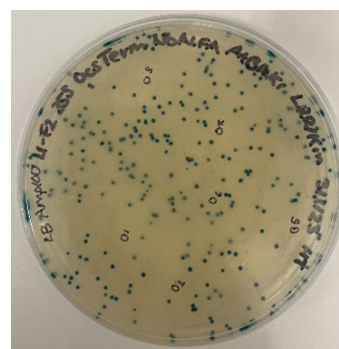
**Helana Trantino**

Williams Group | Supervisor: Carl McCombe and Simon Williams

Nanobodies are selective binding antibody fragments that are becoming an increasingly popular molecular tool. During my time over the summer, I was fortunate enough to work on two nanobody-related projects, one related to plant immunity and the other to nanobody production.



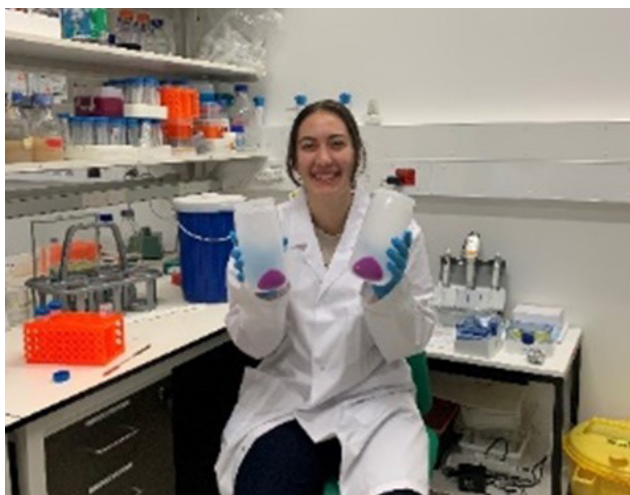
Plants have evolved receptors that trigger an immune response once they detect small pathogen-secreted proteins called effectors. Some of these receptors lack signalling mechanisms and so form complexes with receptor-like kinases (RLKs) such as SOBIR1 and BAK1



that are responsible for triggering downstream immune responses. The focus of my first project was to use complementary nanobodies that bind to the same protein to recruit SOBIR1 and BAK1 to trigger an immune response.



If successful, nanobodies against effectors that the plant hasn't developed an immune response against could be used to engineer plants with novel disease resistance, minimising the detrimental impacts of plant pathogens and increasing food security. I first expressed and purified a pink/purple, fluorescent fusion protein, mCherry-ALFAtag, which would be the target protein of both nanobodies. Using Golden Gate cloning, I fused the nanobodies LaM-4 (for mCherry) and ALFA (for ALFA tag) to SOBIR1 and BAK1 respectively. After sequencing these constructs, a deletion was found in ALFA BAK1 which prevented both nanobody-RLKs from being cloned into the same construct together and agroinfiltrated along with mCherry-ALFA to see if an immune response could be seen.



Simultaneously, I was also focused on optimising an *E. coli* co-expression system called CyDisCo (cytoplasmic disulfide bond formation in *E. coli*) for the production of camelid-derived nanobodies. CyDisCo uses two enzymes, protein disulfide isomerase and sulfhydryl oxidase, that help fold disulphide bonded proteins and increase protein yields. Using Golden Gate cloning I assembled the genes for both enzymes from camels into one construct. The newly termed CyDisCoCam is now ready to be co-expressed with camelid nanobodies to determine if the system can improve yields of difficult to produce nano-bodies.

This program provided the perfect bridge between my previous undergraduate study and my Honours. Not only did I become more familiar with lab techniques but also experiment planning and structuring which will be invaluable in the year to come.

## Does bushfire influence koala diet quality?

**Jackson Rendall**

**Marsh Group | Supervisor: Karen Marsh, Kara Youngentob**

The koala is undoubtedly one of Australia's most beloved and iconic animals. It was a major victim of the 2019/20 mega fires, symbolizing to the public the impact of these fires on Australian wildlife. Despite this, there is

currently limited understanding of how koalas obtain food in burnt landscapes. Post-fire regrowth, termed epicormic growth, has a different nutritional composition to mature leaves, and sometimes a higher toxicity. Despite this, koalas that had survived the fire and its immediate aftermath were seen consuming epicormic growth in burnt habitat. This project aimed to investigate how the diet quality of these koalas compared to koalas with a diet consisting of mature leaves in unburnt habitat. The knowledge gained in this project will enable us to better manage koala populations post-bushfire, ultimately helping to save the species from extinction. For example, it will inform whether rescued koalas can safely be released back into burnt habitat.

My project analyzed the available nitrogen concentration using near-infrared spectroscopy (NIRS) of freeze-dried scat samples (fecal droppings) collected following the 2019-20 fires in burnt and nearby unburnt habitat in the Monaro region of New South Wales. Available nitrogen in scat is an indicator of dietary digestible protein, which is a key nutrient essential for growth, detoxification, and general function. The findings suggest that koalas in the Monaro region are getting roughly equal protein from epicormic regrowth in burnt habitat as they are from mature leaves in unburnt habitat. This helps to explain how koalas in the Monaro region that survived the fire and its immediate aftermath were persisting in burnt landscapes, although more research is needed to know whether this is consistent in other areas.

It was great being able to conduct a larger scale research project, which helped me to understand how tedious yet rewarding scientific research can be. Another great opportunity from this project was being able to help with the fieldwork that the Marsh Group does in the Snowy Mountains setting up acoustic recorders to help understand where in Kosciuszko National Park an elusive and understudied koala population lives. Additionally, coming to ANU from Flinders University and meeting some amazing people who also love biology and research was an amazing opportunity.



Access gates can be difficult to open in Kosciuszko NP. Photo Credit: James Skewes



Ground koala scat prior to scanning with NIRS. Photo Credit: Jackson Rendall

## How does warming affect guppy reproduction?

**Kate Farkas**

Head Group | Supervisor: Megan Head, Joseph Chung

Under high temperatures, guppies adopt a “live fast, die young” way of life, where they require more food intake to grow faster and mature earlier. But when guppies at high temperatures have had a limited diet early in life, how do they compensate for this amongst their fast-paced life? Do they invest more into growth or gamete production, and how does this affect their health and reproductive success? Guppies are versatile and accessible as a model system, and studying them may provide some insight into how rising temperatures may affect freshwater fish populations.

My summer research scholarship was with the Head Group, where I helped run an experiment with Joseph Chung looking at how temperature and diet affects growth and reproduction in female guppies. Alongside two fellow summer scholars, I was helping to rotate breeding pairs between tanks, count babies, photograph fish and measure their body lengths in ImageJ.

As well as helping in the lab, I travelled to Kosciuszko National Park for a project on alpine soil invertebrates. With the help of Prof. Megan Head and the Nicotra Group, I designed and built 60 pitfall traps in the AMRF FutureClim site - an experiment looking at the effects of heat and drought on alpine communities. Hiking through the mountains in bloom and meeting researchers from a different field was a highlight of this summer.

Working in both the lab and the field has shown me two different sides to what a career in biology could look like. I have found a new appreciation for the time and dedication that researchers commit to their work, and I am grateful for the opportunity to contribute to this research at the RSB.

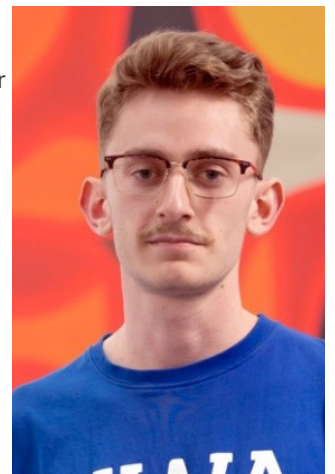


## Protoplast assay for fungal pathogen molecule detection

**Jasper Lee**

Schwessinger Group | Supervisor: Bayantes Dagvadorj, Ben Schwessinger

The objective of the project was to continue development of an assay for detecting fungal pathogen molecules in wheat. It is a crucial project as fungal diseases in cereal crops currently cause severe yield losses worldwide. The information that can be gathered from this new biological assay would greatly assist to develop new strategies to combat plant fungal diseases.



My project involved improving an assay involving the delivery of plasmids into wheat protoplasts, which are plant cells manipulated to survive without their cell walls. The plasmids are modified to contain genes predicted to be responsible for pathogen avirulence and mixed with protoplast preparations from varying varieties of wheat coding different resistance genes. If the introduced fungal pathogen molecule is recognized the corresponding triggered defence response in the wheat cells can be detected using a genetically wired bioluminescent reporter gene. As part of my project I undertook a range of troubleshooting experiments to test alternative genetic sensory components (promoters) and helped to identify two additional promoters of better use in the assay.

The SRS program was really beneficial to me. Not only did I learn a lot about fungal crop diseases, but also about time management and teamwork. Both of my supervisors are great mentors and the whole lab is very friendly. The program is also a great way to make new friends as the RSB does a good job at hosting social events to get to know everyone else in the program.



## Detecting plant pathogen and resistance gene interactions in cereal crops

Lucy Molloy

Rathjen Group | Supervisor: Xiaoxiao Zhang



Worldwide, cereals are important crops; both through direct consumption of the grains and indirectly through consumption of animal products derived from these crops. As such, cereals make up a massive portion of the global caloric consumption. Crop losses from plant diseases are increasing in prevalence, especially with the changing climate. Understanding the natural variation and mechanisms of pathogen resistance in crop is thus integral to introducing resistance into cereals to help address global food security.

Over the summer Elena De La O and I worked on a project aiming to develop an assay capable of identifying interactions between cereal crop resistance and plant pathogen effector genes. The assay design seeks to ascertain which resistance genes are able to recognise and confer resistance to specific pathogen effectors. Our project used molecular cloning techniques to successfully create plasmid vectors containing resistance and effector genes and the regulatory genetic elements appropriate for gene expression. These constructs were transiently expressed in the model plant *Nicotiana benthamiana* and also tested in a split luciferase wheat protoplast assay. Both assays endeavour to detect plant defence activation resulting from effector-resistance interactions and thus identify resistance gene(s) confer immunity against a specific effector.

Being able to participate in this ongoing project has been a valuable experience, to gain both insight into how research is conducted, and meet some great and passionate academics, students and support staff. This opportunity has been very rewarding and is very different to what I have experienced in undergraduate courses.

## Decrypting C<sub>4</sub> chloroplast-nuclear signalling networks

Riley Furbank

Chan Group | Supervisors: Kai Chan, Suyan Yee

High light, heat, and drought stress are becoming more frequent and extreme with climate change. While most of our staple crops (C<sub>3</sub> plants) perform sub optimally in these extreme growth conditions, plant species with more sophisticated C<sub>4</sub>-photosynthetic physiologies appear more tolerant to these abiotic stresses. In C<sub>3</sub> plants the chloroplast has been established as the environmental sensor of the cell, detecting abiotic stresses such as high light and through reactive oxygen species or secondary messenger molecules communicating this to the nucleus to trigger appropriate changes in gene expression. In contrast, little is known about such retrograde signalling pathways in C<sub>4</sub> plants whose dimorphic physiology include cell-type specialised chloroplasts. In my project I used the model C<sub>4</sub> plant *Setaria viridis* to investigate stress-related changes in gene expression to determine whether a singlet-oxygen signalling pathway operates differently across the specialised cell types of C<sub>4</sub> plants.

*S. viridis* plants treated with beta-cyclocitral (an inducer of the singlet oxygen retrograde signalling pathway) were exposed to high light and heat stress conditions. Leaf fractions enriched in either mesophyll or bundle sheath cells were prepared and their mRNA purified for real-time quantitative PCR (qPCR) to assess changes in the relative expression of retrograde signalling pathway genes across both treatments and cell types.



## How nutritious is burnt koala habitat?

Mina Kearns

Marsh Group | Supervisor: Karen Marsh, Kara Youngentob

This summer I enjoyed getting a taste of what research is like both in the lab and out in the field. I spent most of my time helping PhD student Murraya Lane compare the nutritional content of eucalypt leaves in burnt and unburnt habitat areas after the 2019-2020 bushfires with the aim of helping inform koala population recovery and management post-fires.

Epicormic tree growth after a bushfire produces leaves with differing nutrient compositions to the leaves from unburnt trees. During my internship I helped prepare leaf samples (grinding) and analyse their nitrogen (a proxy for protein) and plant secondary metabolite (PSM) contents using near-infrared (NIR) spectrometry. I found large variation in the response of different tree species to bushfires, with some having more nutritious epicormic leaves, some having more nutritious mature leaves and others showing no difference in available nitrogen between the leaf types. The NIR spectra also suggest that PSM concentrations are even a bit lower in epicormic leaves, meaning that these leaves are easier for koalas to eat.



Over the summer I enjoyed getting hands on experience helping with many stages of koala/nutrition studies and becoming intimately familiar with the scent of various *Eucalyptus* leaves. The most valuable thing I'll take with me from this internship, however, is the perspective of research not just from the helper but from the researcher. When I asked some other members of the lab what the best part of their honours or PhD was, they told me it was the sense of ownership over a project that was fully theirs, and that after working on it for so long it's like their 'baby'. Throughout my degree I had one foot in the door about whether or not I wanted to pursue further research, but after this summer I think I will give it a go!

## Differentially expressed anthocyanin pathway genes in *Caladenia carnea*

You Jin (Angelynne) Chang

Peakall Group | Supervisors: Darren Wong, Rod Peakall

The commonly known spider orchid, *Caladenia* (*Orchidaceae*) is a highly diverse genus with more than 350 species of orchids. My project focused on a native food deceptive orchid species, *Caladenia carnea* where the flowers have evolved pollination strategies such as colour display to potentially impact pollinator preference and visitation. Flowers of *C. carnea* are typically pink but white morphs are also present in natural populations. This project aimed to elucidate the



key anthocyanin pathway genes underlying the floral colour shifts between the morphs.

My Next Generation Sequencing (NGS) bioinformatics pipeline analyses included filtering low quality reads with *fastp* (adapter trimming tool), performing *de novo* transcriptome assembly with *Trinity* and conducting open reading frame (ORF) prediction with *EvidentialGene*. *Bowtie2* was then used to align reads with local alignment mode to the reference genome and *featureCounts* employed to quantify transcripts. Differential expression analysis of RNA-seq was then performed with the read counts using *DESeq2* and *EdgeR*. For the functional annotation of transcripts, *Diamond* was applied to align transcripts against the UniProt protein reference database followed by annotating the transcripts with the UniProt annotation file. From my analyses I identified 34 essential anthocyanin pathway genes potentially implicated in the floral colour shifts between the morphs.

My project also include fieldwork, including locating *Caladenia congesta* on Black Mountain, a species uncommon to the ACT (e.g.). Overall the summer research experience has been invaluable, providing a fantastic opportunity for me to explore the natural beauty of Australia while also getting the chance to further develop my coding skills in R, Bash and Python. A summer well spent!

## Summer of Love - keeping up with guppy and seed beetle reproductive rates

Rozita Higgs

Head Group | Supervisor: Megan Head, Meng-Han Chung

In the Head lab this summer there was no shortage of projects. My time was divided between the aquaria and entomology labs where myself and two other summer scholars (Yusheng and Kate) worked to keep hundreds of guppies and beetles alive and loving.

My task was to grow and maintain the lab population of *Megabruchidius tonkineus*, a species of seed beetles recently found on ANU campus. Though an invasive species in Europe, little is known about their distribution in Australia. I discovered the beetles prefer to lay eggs on the seeds of *Gleditsia tricantha* (Honey Locust





trees) - which is slightly unhelpful given seeds are not available year-round. I observed a generation time of around 8 weeks which could be shorter in the wild and with higher humidity. The ultimate goal of the research is to understand the beetles courtship display, mating rejection and the relationship between sex ratio and sex role reversal. There is much to get excited about once the lab population is re-established in June-August when seed pods return to *Gleditsia tricantha* trees across campus.

The other half of my internship has been spent assisting the lab's research into food restriction and water temperature on compensatory growth in female guppies. This involved checking the 430 tanks each morning for little baby fish, taking photos of them, measuring their body length and rotating the adult females through mating tanks weekly. It did not take long for Kate, Yusheng and I to perfect the tasks and start having a competition for the largest number of baby fish found in a single morning (record goes to me with ~100 on Friday the 6th of January). This research is still in the height of data collection, and I am keenly looking forward the results.

## Detecting fungal pathogen molecules in wheat

Ziliang (Raymond) Chen

Schwessinger Group | Supervisors: Benjamin Schwessinger

The impact of fungal pathogens on crop yields pose a significant cost to farmers with fungicide treatments having a devastating impact on the environment. By better understanding fungal pathogens resistance we may improve the disease resilience and yields of crops and reduce fungicide use. To address this my project sought to investigate the interaction between avirulence (Avr) genes from pathogens and resistance (R) genes found in wheat



to better understand plant disease immunity. This was achieved by transfecting wheat protoplasts (cells without cell walls) with plasmids coding a luminescent Avr/R interaction detection system. In wheat cells undergoing Avr/R interactions programmed cell death occurs resulting in loss of luciferase activity, detectable with a plate reader, allowing for a high throughput comparison of different Avr/R gene combinations. This reported system developed in this project can now be used to test, and discover, the interactivity of more Avr/R combinations



My personal highlights of the SRS program were that I got to meet all sorts of people studying in different fields, and learning their stories broadened my knowledge about all kinds of research in Biology. I also enjoy the daily lab work; it allowed me to know how a lab operates and the importance of controls when conducting experiments. This opportunity tested the skills and knowledge I have accrued during my undergraduate studies.

## To burn or not to burn: The effects of fire on Greater Glider diet

Ryan Adams

Marsh Group | Supervisor: Karen Marsh

This summer I've had the pleasure of working within the Marsh Group on their work with eucalypt folivore diet and nutrition. I predominately helped PhD student Melina Budden on her project to better understand the effect of fire on the diet and food quality of Greater Gliders (*Petauroides volans*) - small, nocturnal marsupials endemic to Eastern Australia. Unfortunately, Greater Gliders are recognised as endangered at both a State and Commonwealth level, especially after the 2019-20



bushfires ravaged much of their forest habitat. More frequent extreme fire events are predicted as anthropogenic-driven climate change alter the fire regime of our native forests necessitating the need to determine the impact fire have on Greater Gliders and

their food.

My project helped in preparing pre and post-fire leaf samples collected by Dr Benjamin Wagner for nutritional composition analysis (such as nitrogen content). This data will ultimately be correlated with surveys of the glider populations at each site.

This work marked my first time in a laboratory setting, and what a wonderful time it was. I am thankful for the SRI program providing me with a fantastic primer to the world of Ecology and Evolution laboratory research and career opportunities. Everyone in the lab was wonderful and made the summer fly by. Specifically, I am thankful for the opportunity to experience one of the cushiest weeks of field work one could ask for.



My project examined the variation in the accumulation of the carotenoid derived secondary metabolite,  $\beta$ -cyclocitral (b-CC) in leaves of high-light-stressed versus unstressed *Setaria* ( $C_4$  plant). I used gas chromatography coupled to mass spectrometry (GC/MS) to detect b-CC and uncover how its accumulation differed across whole leaves, and between the mesophyll (M) and bundle sheath cells (BSC). The GC/MS results indicated higher b-CC levels under high-light stress than in unstressed conditions. This supported our hypothesis, as a lower Fv/Fm reflects a downregulation of photosynthesis caused by stress. Interestingly, BSCs produced high levels of b-CC, especially under high light. This is strange because BSCs do not contain photosystem II (PSII), which is responsible for capturing light and containing PSII. In the future, our GC/MS results need to be normalized to the internal standards and to the tissue weights to ensure the observed amount of b-CC is not inflated. Examining the concentrations of other chloroplast stress signals, like PAP and  $H_2O_2$  would also be beneficial.



## Decrypting chloroplast signaling in $C_4$ plants

Sona Jerry

Chan Group | Supervisors: Kai Chan

Climate-related stresses like drought and heat are significant threats to plant production and food security. Plant chloroplasts detect external stress stimuli and initiate intracellular signaling by producing secondary metabolites and reactive oxygen species (ROS). However, much is still unknown about chloroplast-to-nucleus retrograde signaling in plants with  $C_4$ -photosynthetic physiologies. Therefore, improved understanding is crucial to developing stress resilient crops.

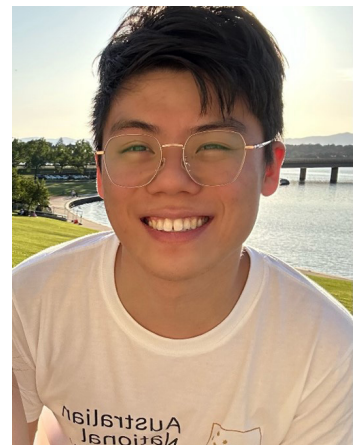


## The impact of environmental change on female guppies

Yusheng Wang

Head Group | Supervisor: Megan Head

The guppy (*Poecilia reticulata*) is one of the world's most widely distributed tropical fish and one of the most popular freshwater aquarium fish species. Accelerated increases in the weight or length of guppies following a period of slowed development (e.g. during nutrient deprivation) is termed compensatory growth. My study sought to examine the impact of temperature (26°C versus 30°C) on the compensatory growth in juveniles reared initially on normal or restricted diets and the long-term effects on adult fitness. The project involved monitoring the growth, lifetime reproductive success and body condition of adult females, including body length, cellular immune response, gut length, total egg number, number of fertilised eggs, and relative telomere length.



Although the research is ongoing, we hypothesise that at each temperature the control-diet juveniles will grow bigger than their low-diet counterparts during the period



of food restriction. After their diets are back to normal, we expect low-diet individuals will have faster growth rates which will allow them to reach the same adult size as the control fish, though likely taking longer to fully mature. As rapid growth can cause accumulation of oxidative stress and impair body function, we expect that fish experiencing compensatory growth will have lower adult fitness in particular in those reared at higher temperatures. This study aims to figure out how food availability and temperature together can impact fish health and reproductive fitness.

My personal highlight from this Summer Research Internship is having my first research experience at ANU. I had a chance to conduct independent ecological research, from which I learned a lot. Overall, this experience is a treasure for my future research career. I would highly recommend it.



**ANU Research School of Biology  
2022 Summer Research Scholars**

The RSB 2022/23 Summer Research Program had 25 participants from around Australia and New Zealand