Australian Government

Department of Agriculture, Water and the Environment

Phenology, demography and distribution of Australia's fruit flies

“Phenology, demography and distribution of Australia’s fruit flies” is funded through the Strengthening Australia’s Fruit Fly System Research Program of the Department of Agriculture Water & Environment, with matched funds from State and Territory governments

Phenology, demography and distribution of Australia's fruit flies

Successful pest management relies on three core pieces of information:

1. seasonal cycles affecting pest activity (phenology)
2. reproductive patterns and population changes (demography)
3. where the pests are (distribution)

To strengthen pest management we are mining existing data and generating new data sets:

1. Lots of good research has been done in the past but it has been regional or state based
2. This project is a national project with 13 separate but linked activities
3. It ha33s also been a capacity building project with many of the 70+ staff (permanent, temporary and casuals) never employed in fruit fly research before.

Funding was 50% DAWE and 50% States and Territories

Project Management Team

Peter Leach	Project Lead, QDAF
Tony Clarke	Scientific Advisor and QUT Lead
Penny Measham	Project Coordinator, QDAF
Solomon Balagawi	New South Wales Lead Researcher
Brian Thistleton	Northern Territory Lead Researcher
Peter Crisp	South Australia Lead Researcher
Mark Blacket	Victorian Lead Researcher
Touhidur Rahman	Western Australian Lead Researcher
Lara Senior	Queensland Lead Researcher

Activity Leads	
Natalia de Souza QDAF	Activity 2. Carry out demographic studies
Lara Senior QDAF	Activity 3. Quantify host usage
Hoan Le QDAF	Activity 4. Data for day-degree models
Vesna Gagic QDAF	Activity 5. Collate and analyse field phenology data
Brian Thistleton NTDITT	Activity 6. Host availability as drivers of population peaks
Tony Clarke QUT	Activity 7. Competition as a driver for demography
Shahrima Tasnin QDAF	Activity 8: Detailed phenology & demography of *B. tryoni*
Solomon Balagawi NSWDPI	Activity 9. Distribution mapping, modelling and risk analysis
Stef De Faveri QDAF	Activity 10. Lure response
Mark Blacket DJPR VIC	Activity 11. Metabarcoding
Mark Blacket DJPR VIC	Activity 12. LAMP in-field diagnostic tools
Penny Measham QDAF	Activity 13. Collate information

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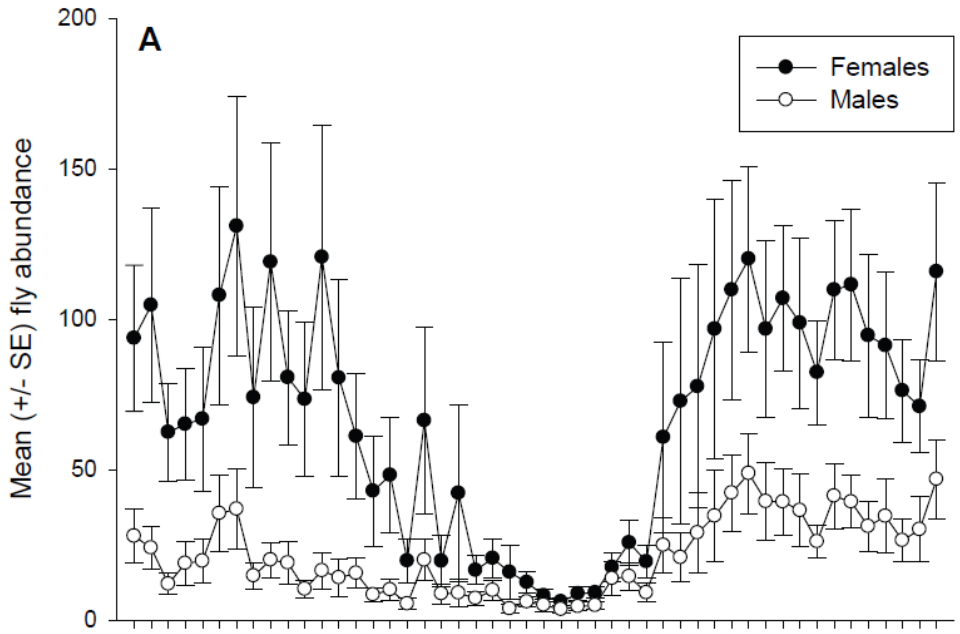
Vivian Sandoval
Alexandra Ainscough
David Nelson
Georgia Schleehauf
Linda Joya
Liz Hall

Phenology, demography and distribution [and diagnostics] of Australia's fruit flies

What is phenology?

- The study of repeatable change in a biological system over time, commonly at a yearly cycle
  - e.g. the fruiting phenology of a crop
- In the current project we've been looking at the changes in population abundance of fruit flies over the year



What is demography?

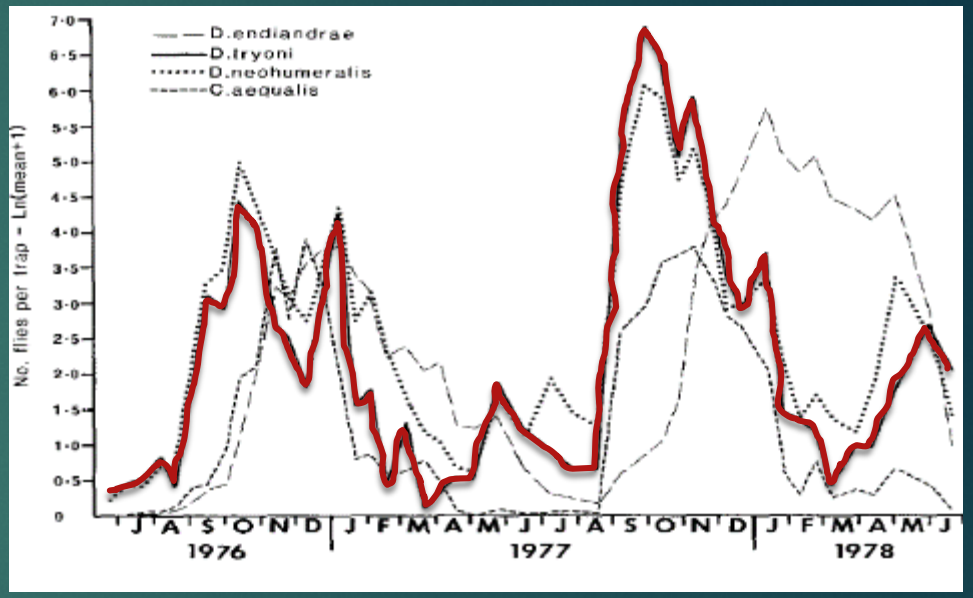
- Demography describes a group of individuals by their numbers, age and [normally] reproductive potential
- A population's demography impacts it's subsequent population dynamics
  - A predominantly young population will have high reproductive capacity and so grow; a predominantly old population will have low reproductive potential and so decline.

Why phenology & demography together?

- Phenology and demography are two sides of the same coin and each informs the other.
- Robust phenological and demographic understanding is essential for accurately predicting future populations.
- Being able to predict populations supports:
  - The timing of on- and off-farm controls
  - Optimising the best control to be using at a given time
  - The use of winter windows for market access
  - Accurate reinstatement dates they are neither too long nor too short
  - Predicting good versus bad fruit fly years

Key questions

- How variable are Qfly dynamics around the country (i.e. tropical vs subtropical vs temperate)?
- Why, in many areas, are fly populations depressed even though temperature and host are not limiting?
- How many generations are there a year?
- What drives the very rapid spring build-up?
- What drives year-to-year variation?
- What is the role of host?



Approach

- Complex, multifaceted, and generally hugely logistically demanding, involving all members of the project
- Many components applied to all our target species, some just to *B. tryoni*
  - Collation and analysis of historical trap data (all species)
  - Constant temperature studies and day-degree models (all species)
  - Laboratory-based life-table studies (all species)
  - Field-based population demography (Qfly only)
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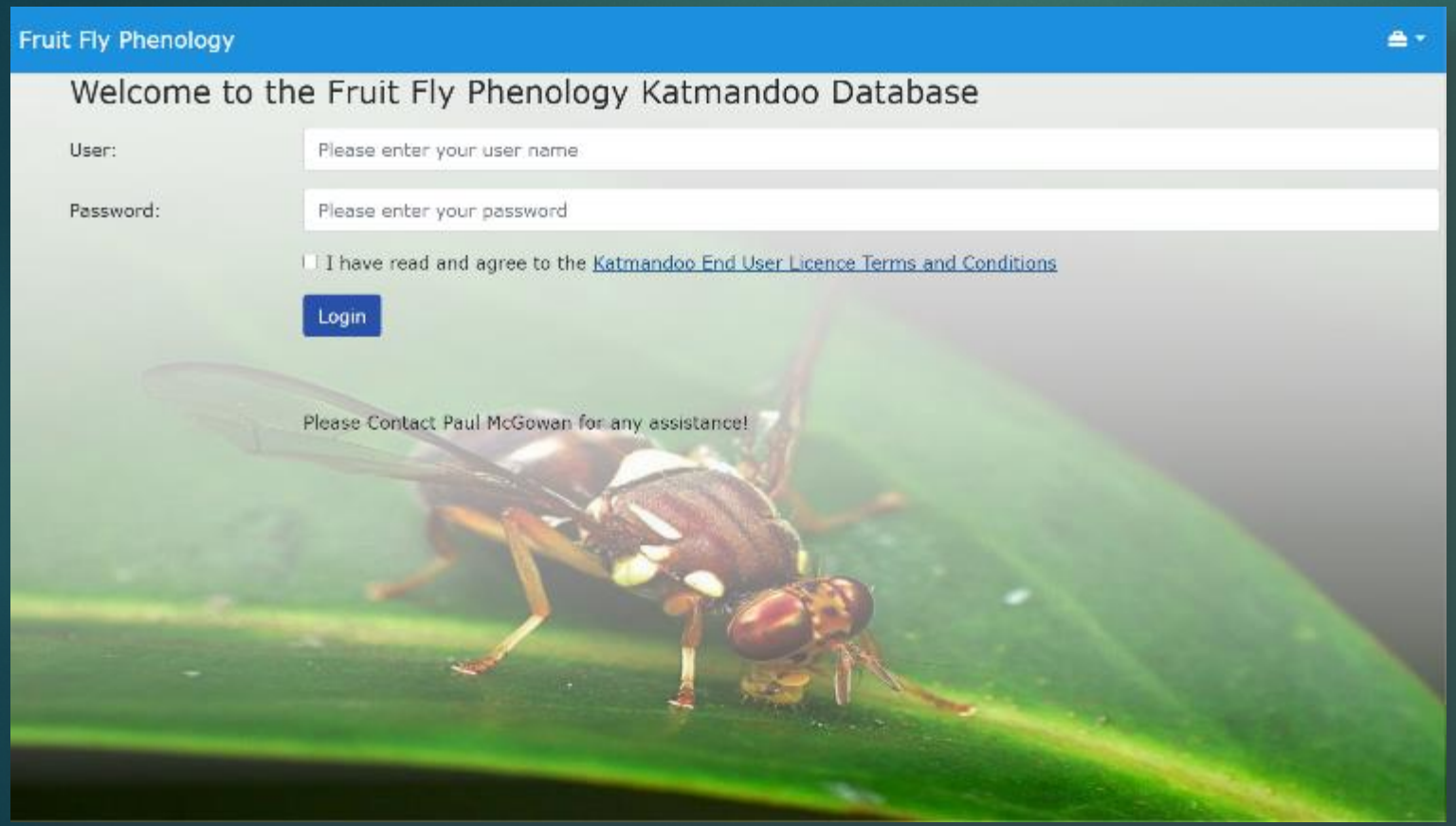
Trapping database



- Captures all available Australian fruit fly trapping data sets

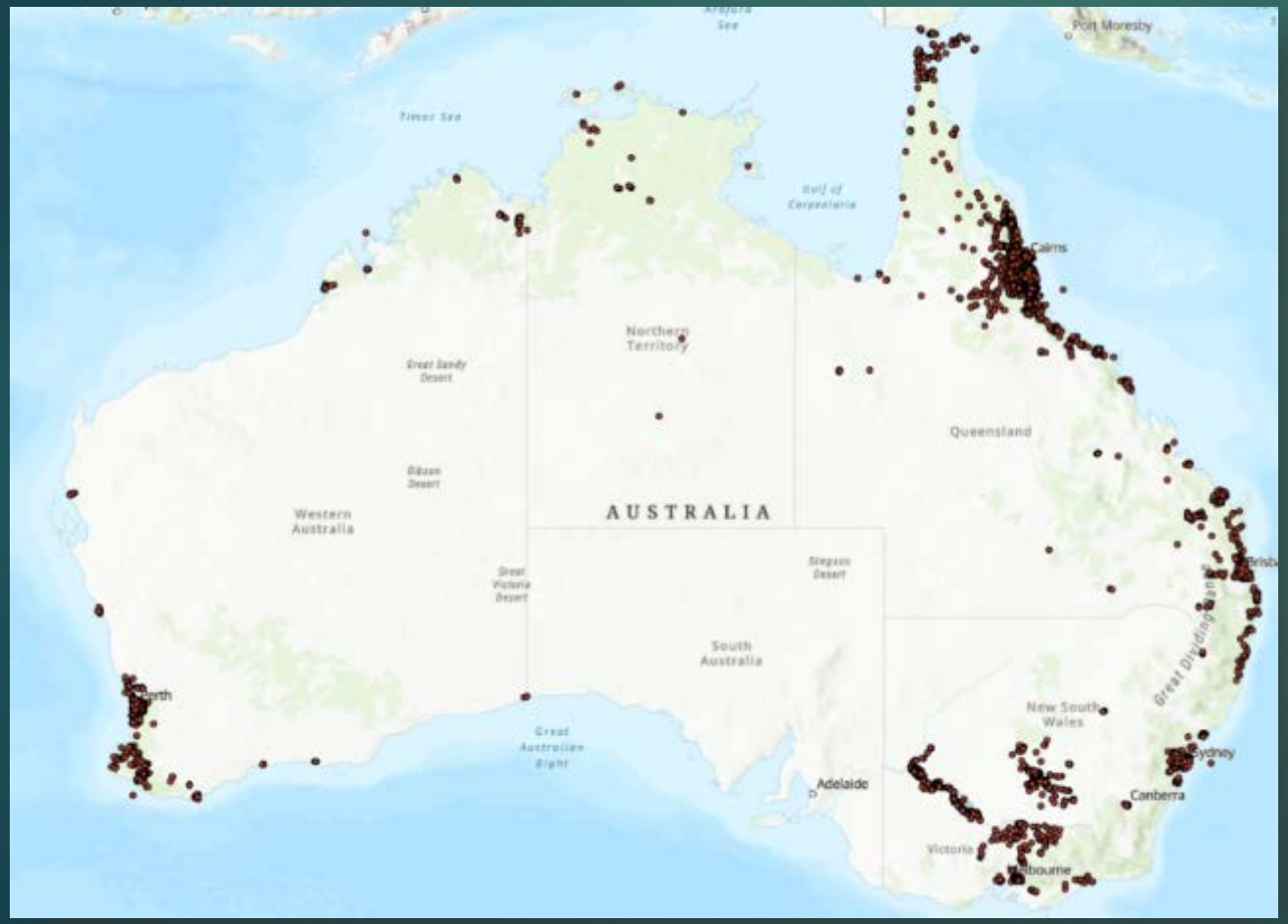
  > 2.1 million lines of data

- Improved data recording in the future and use for trade negotiation purposes (DAWE)

Slide contribution Vesna Gagic

South Australia's traps have **not** been included in the mapping work

Distribution of the traps



- 15,072 traps
- 2,113,421 individual samples
- 1944-1959 and 1992-2021

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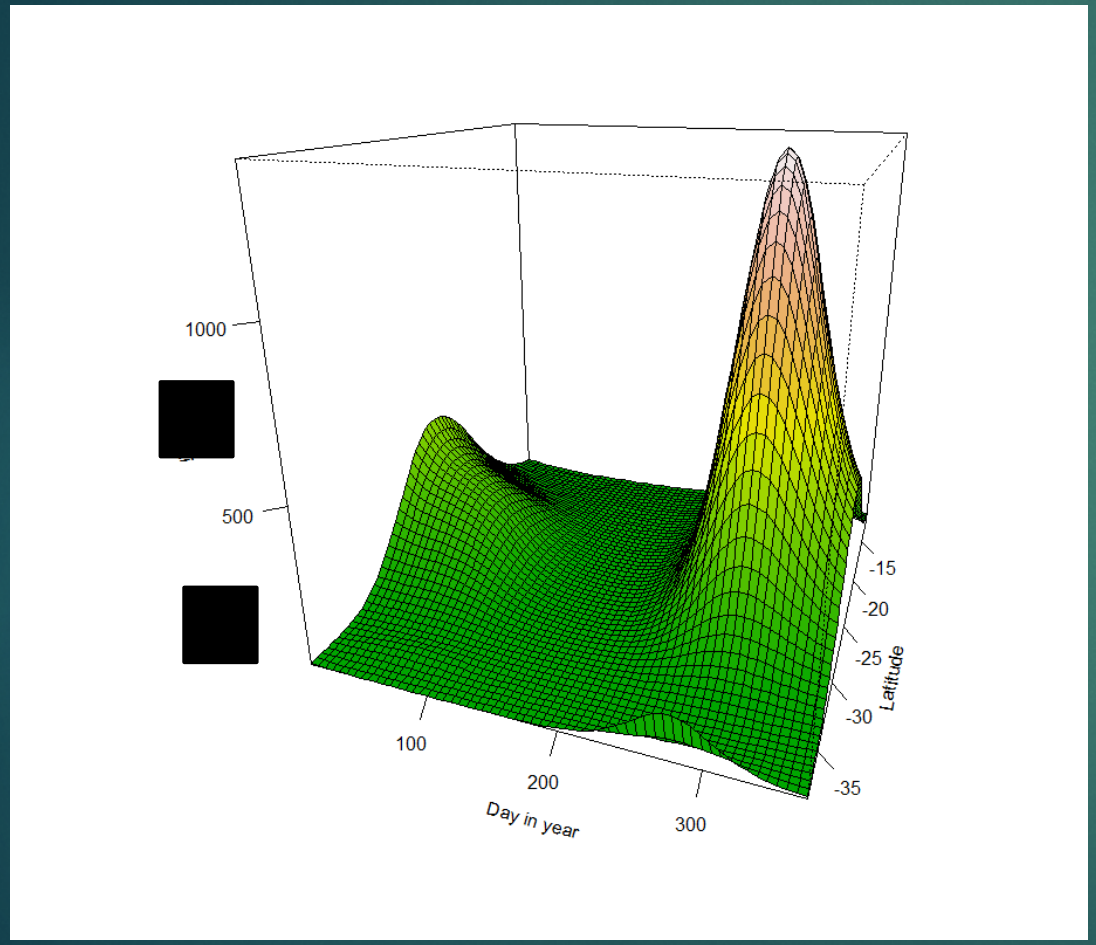
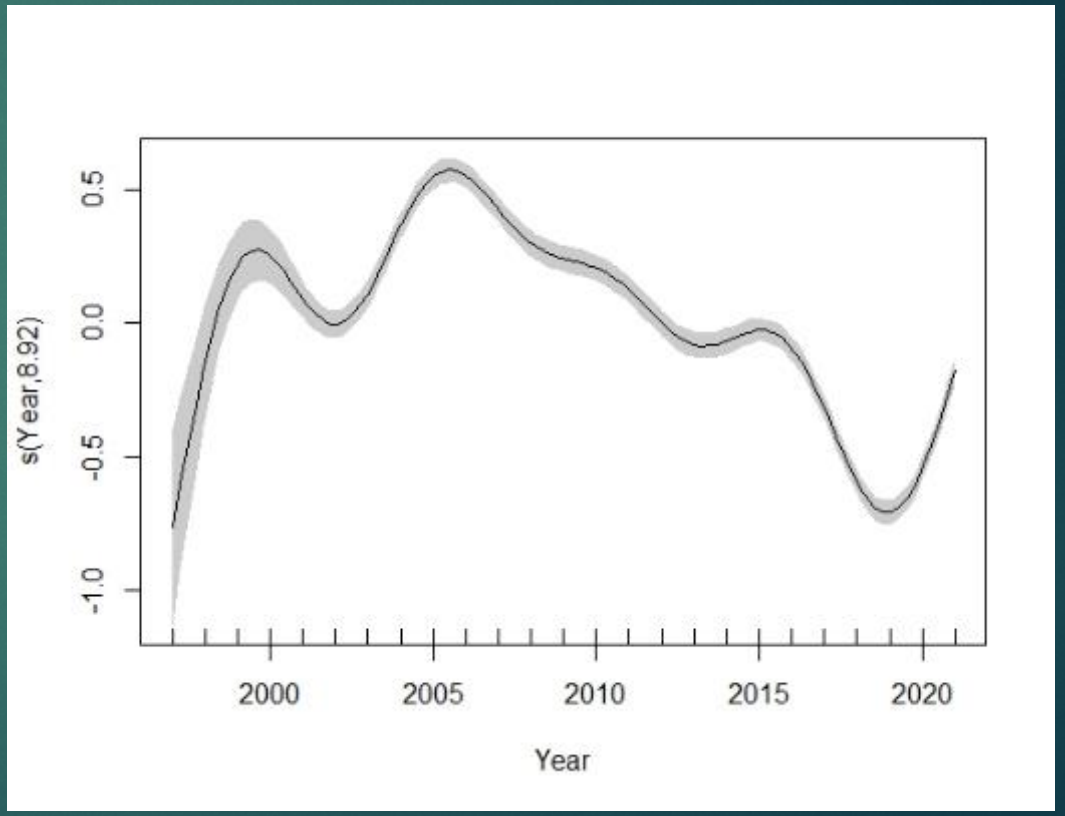
Short and long-term cycles





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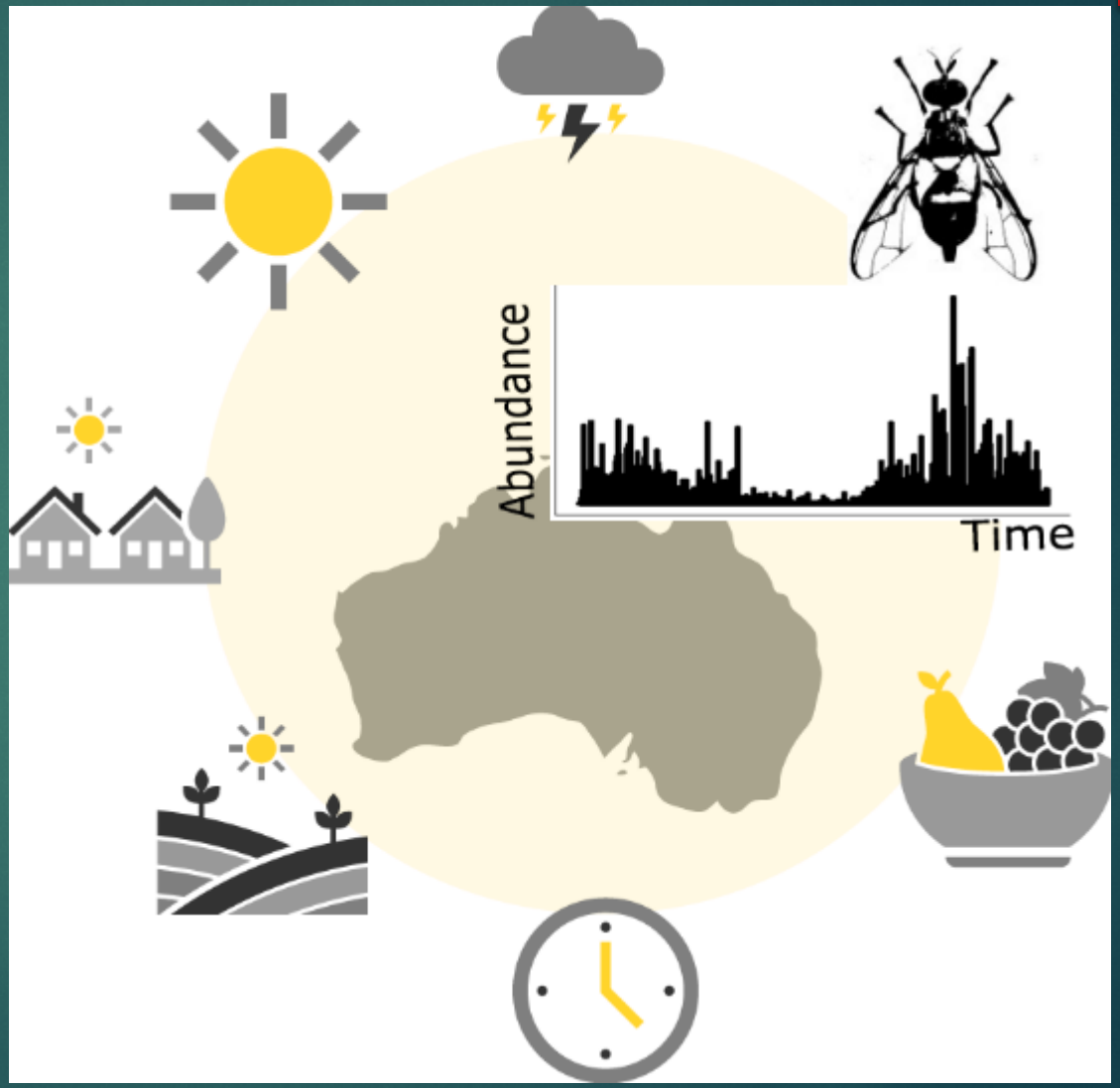
Environmental drivers



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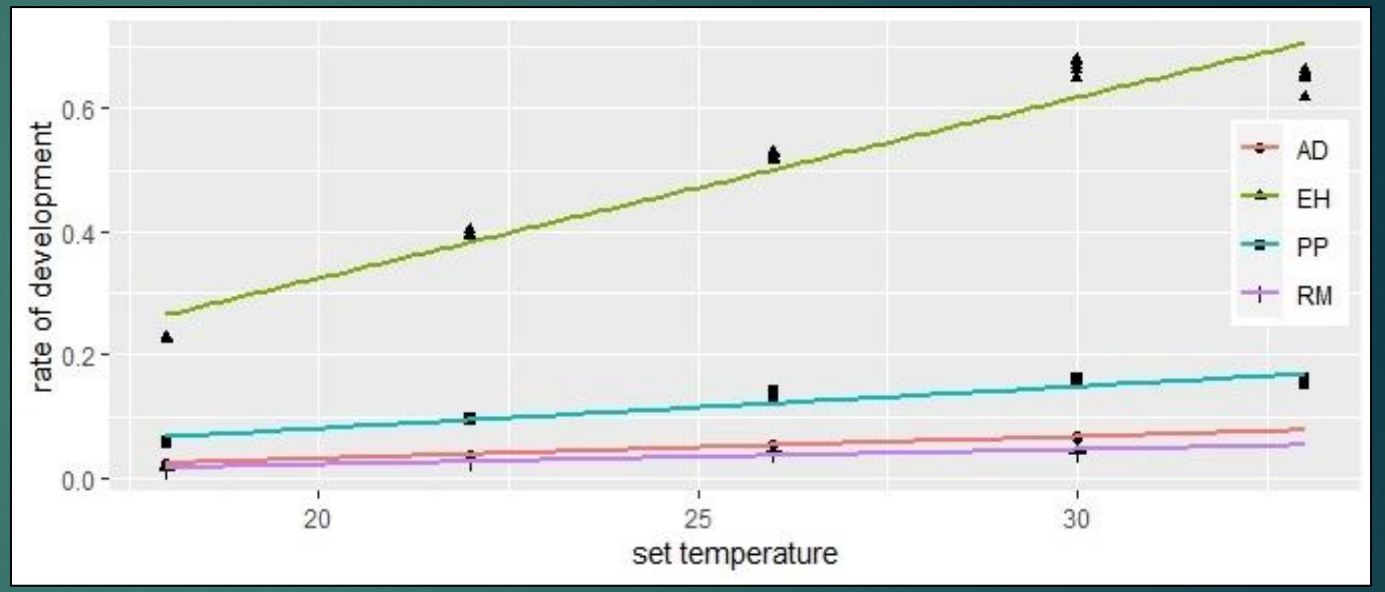
Day degree models

- Hugely logistically demanding
- Four life-history stages * six fly species * three developmental substrates * five-six constant temperatures * 5 replicates
- Life-history stages:
  - time to egg hatch
  - larval developmental time
  - pupal development time
  - time from adult emergence to first egg lay



Slide contribution Hoan Le

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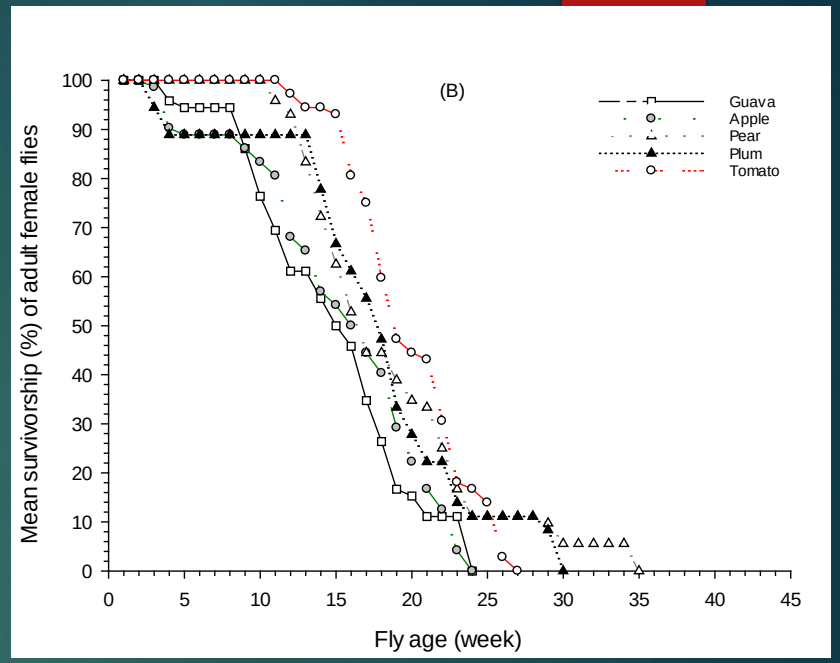
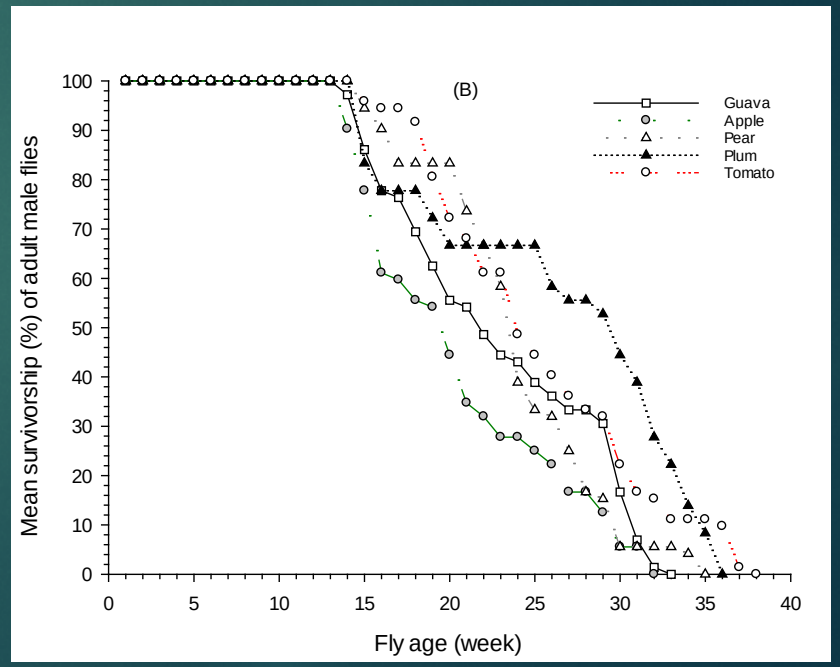
Life-table studies

- Measures age-specific survival rates of all life stages on different hosts
- Records life-time egg production for adult flies reared on different hosts
- This data is used to develop stage-based life tables of each fly species

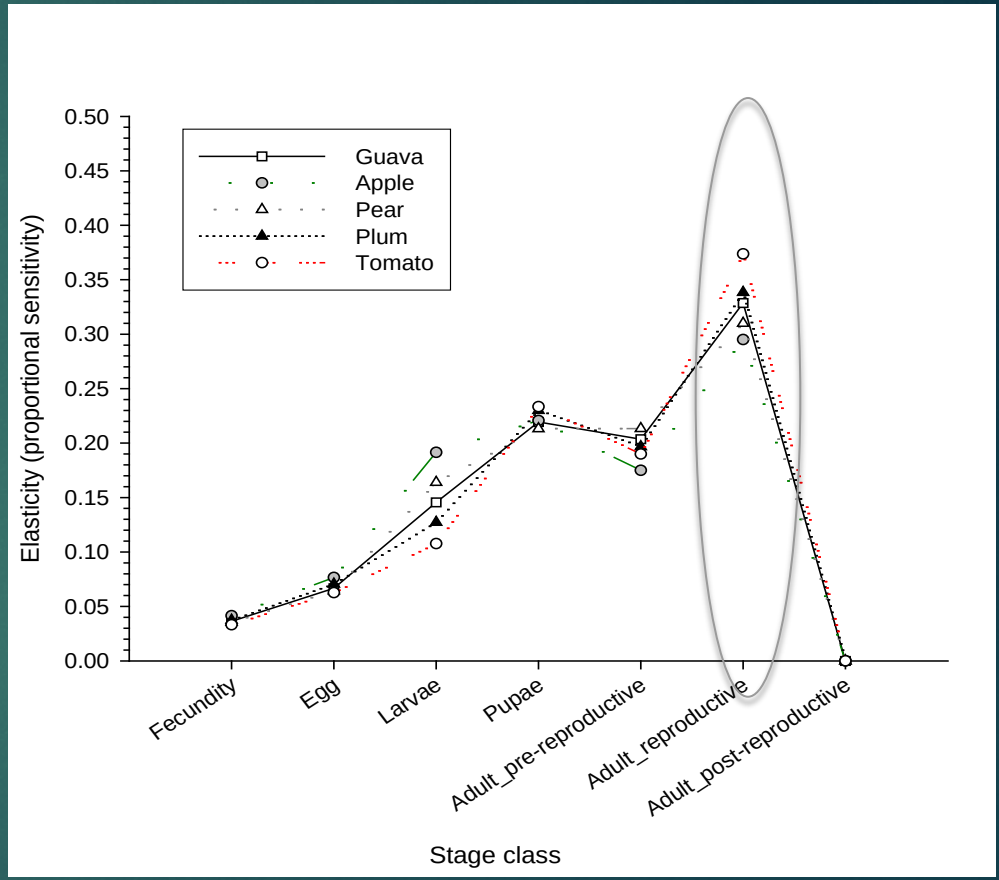




Slide contribution Natalia Souza & Solomon Balagawi

Example of application

- Determine the sensitive life stage of the population and then target the sensitive life stage for management intervention
- Provide direction on what management strategy to use
  - Management strategy targeting adult reproductive stage, e.g. SIT, Protein bait, MAT



Sensitivity of population growth rate of Qfly at different stage-class from five host fruit

Slide contribution Natalia Souza & Solomon Balagawi

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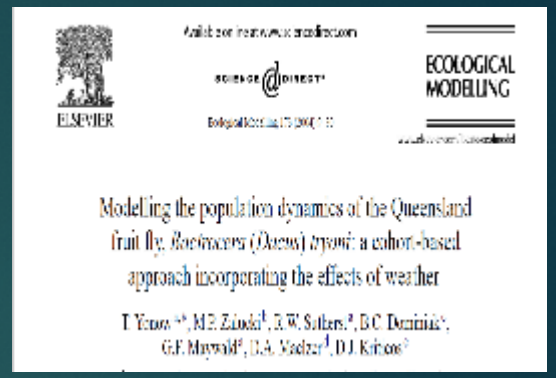
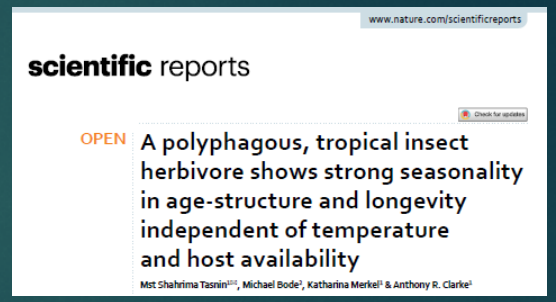
Field demography and seasonality

- "*Until the mechanisms determining the onset and the ending of over-wintering are understood, it will be difficult to improve the model predictions of fly numbers from about May to August*" (Yonow et al. 2004)



- *"Contrary to expectations, the population of B. tryoni at our* [subtropical] *site during the year was composed of only one or two, very occasionally three, generational age-groups.* [There was] *a gap in population growth from mid-autumn to late winter. Notably, the "autumn to winter" breeding cessation is not temperature driven, as the lower temperatures are not cold enough to stop breeding. Breeding at the site was also not halted due to lack of host fruit for larvae, as fruit are continuously available at that site."* (Tasnin et al. 2021)



Questions

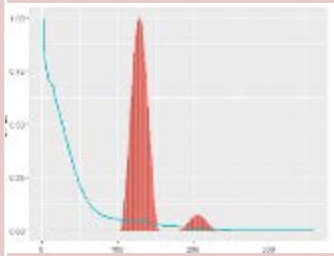
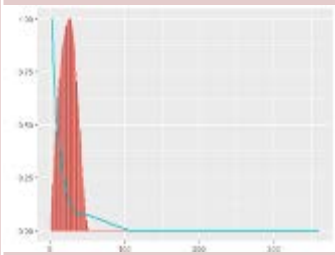
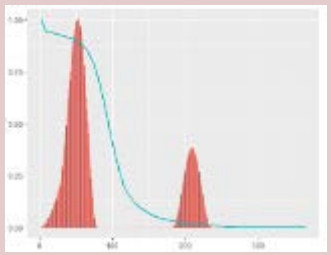
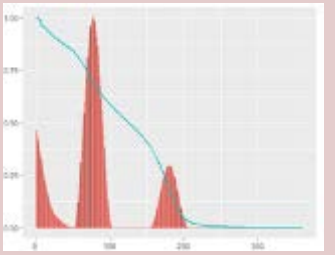
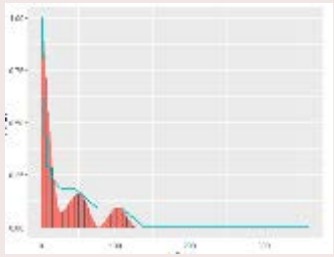
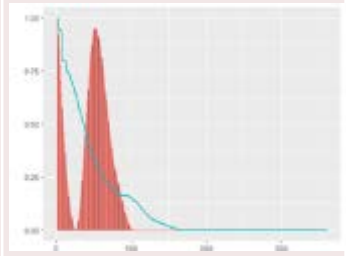
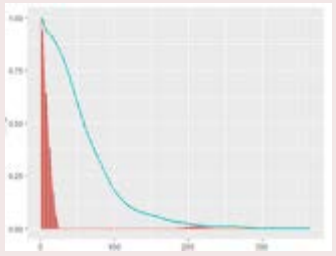
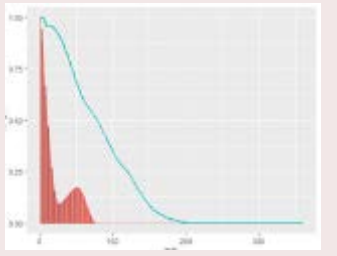
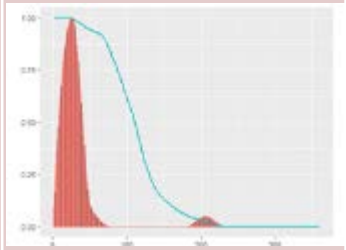
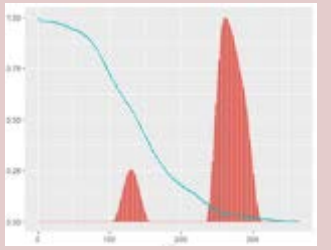
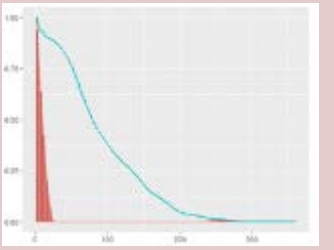
- Why does Queensland fruit fly shows the overwintering depression in population abundance?
- Does females breeding activity changes during the time of the year?
- Is there an endogenous clock mechanism that is driving the phenology?

Method: Age-structure estimation

- Captive cohort: unknown age adult flies collected from field using various lures and traps
- Reference cohort: known age fly emerged in the lab from various wild collected infested fruits
- Both cohorts reared in the laboratory under optimum conditions until death
- Mortality data from both cohorts used to estimate the age of wild flies (Carey et al., 2007, 2008)

Slide contribution Shahrima Tasnin

Result: Age-structure of males

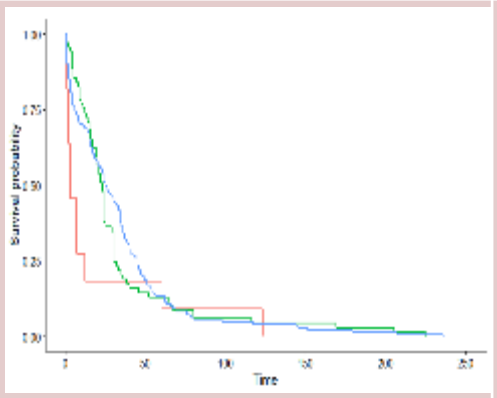
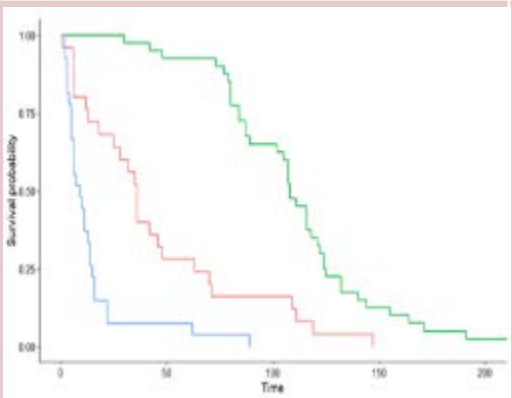
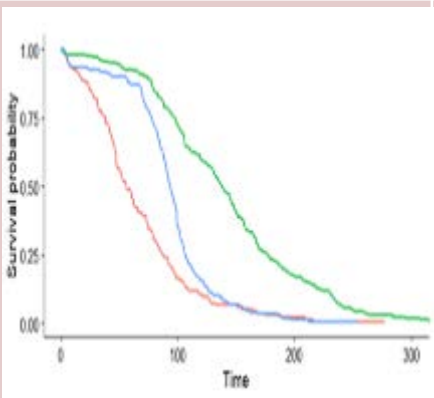
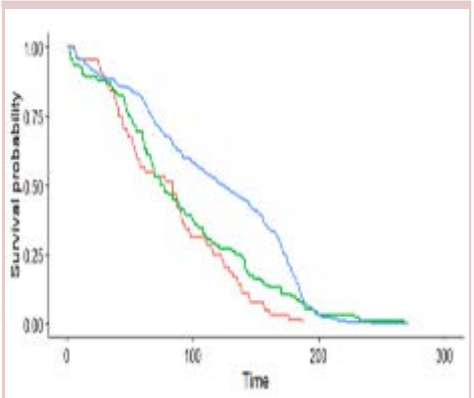
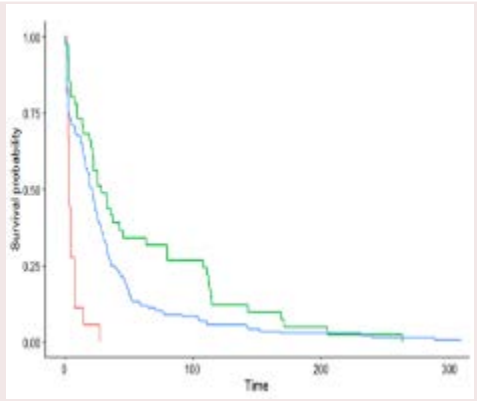
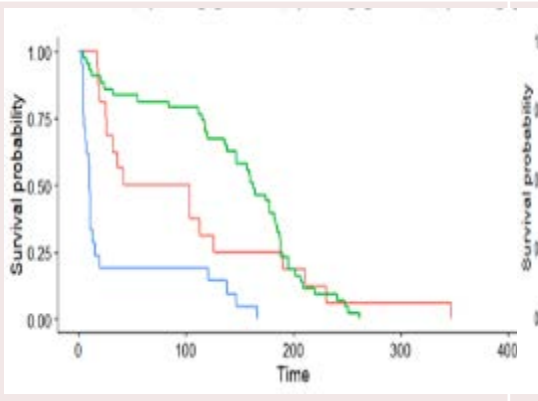
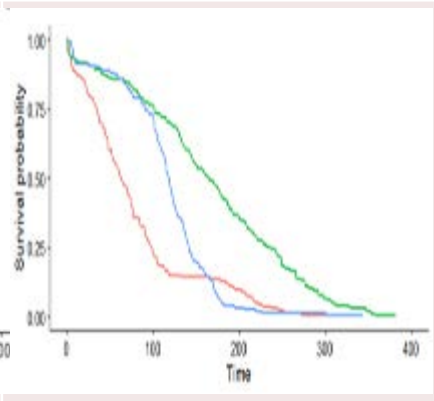
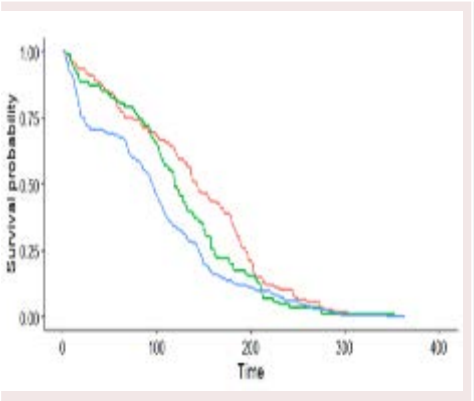
Locations	Mildura, VIC	Ourimbah, N.S.W	Brisbane, South-east QLD	Mareeba, Far-north QLD
Late November 2020				
Late January 2021				
Late March 2021				

Blue line- Survival function of reference fly, Red curve- age-distribution of captive fly

Slide contribution Shahrima Tasnin

Survival function of reference flies

Blue line- late November
Red line- Late January
Green line-Late March

	Mildura, VIC	Ourimbah, NSW	Brisbane, South-east QLD	Mareeba, Far-north QLD
Males				
Females				

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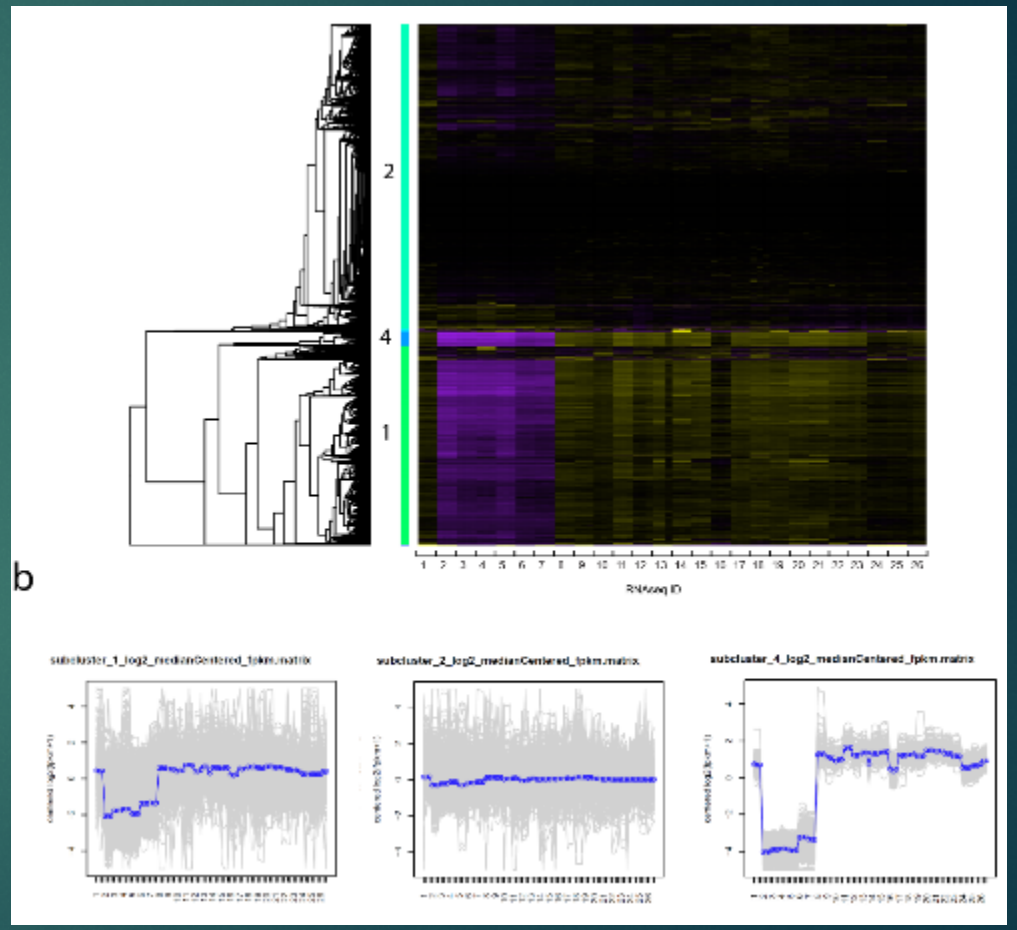
Year-long comparative transcriptomics

- To support the research on seasonality, particularly the ‘winter’ break in breeding
- 78 replicated RNAseq libraries was generated for 26 timepoints spanning one year and 15,646 genes were found to be expressed in total across all samples.
- Analysis ongoing, but clear and dramatic evidence for a genetic shift from reproduction to somatic maintenance in Feb/Mar.



Slide contribution Chloe van der Burg

Summary

- Collection of data only very recently completed, and in some cases is still ongoing, for all activities
- Current focus on finalising data sets and full analysis
- There is accumulating evidence for an endogenous adult reproductive diapause, probably associated in an evolutionary context with monsoonal weather patterns and host availability.
- But the seasonal triggers for that, and how it might be modulated by local, exogenous inputs (temperature, rainfall, host?) still very unclear.
- The datasets collected by the project, once pulled together and assessed as a whole, will help answer some of these questions.

Fruit fly competition & population consequences

- Fruit flies compete with each other over fruit: females compete through aggressive interactions to maintain oviposition sites, while larvae within fruit compete through scramble competition.
- A competitive hierarchy exists across genera, with *Bactrocera* species more competitive than *Ceratitis* species, which are more competitive than *Anastrepha* species. Within a genus, species are also more or less competitive than others.
- More competitive flies can displace less competitive species in time, in space, and in host usage.

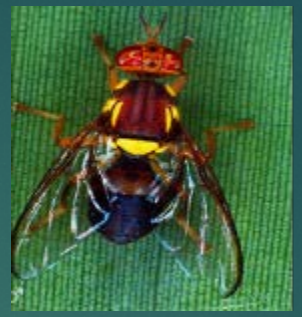
Fruit fly competition & population consequences

- Based on a review of the extensive international literature it is concluded that:
  - If Oriental fruit fly invaded Australia it would out compete and displace all existing fruit fly pest species in Australia, with the exception of *Zeugodacus cucumis* (because of different host usage)
  - If Qfly established in southern WA it would displace Medfly as the major pest species, but is not expected to drive Medfly to extinction
  - In areas where Qfly is now endemic, the permanent establishment of an invasive Medfly population is not expected.
  - There is no literature to say how Spotted Wing Drosophila might be influenced by competitive interactions

Phenology, distribution and diagnostics

- Regardless of the presence or absence of diapause mechanisms, fruit flies are still ectotherms and their development is strongly impacted by temperature.
- With climate change, species' distributions may be moving further south, particularly species which already had subtropical distributions.
- If this is the case it is important to know, and so the project included a sampling component.
- As a fundamental element of sampling is identifying the catch, the project then further incorporated a diagnostics component, which was a priority activity under the DAWE roadmap.

Distribution mapping



Rationale

- Appropriate fruit fly specific management applied
- Sultana et al. 2020 – range may be extended

Aim

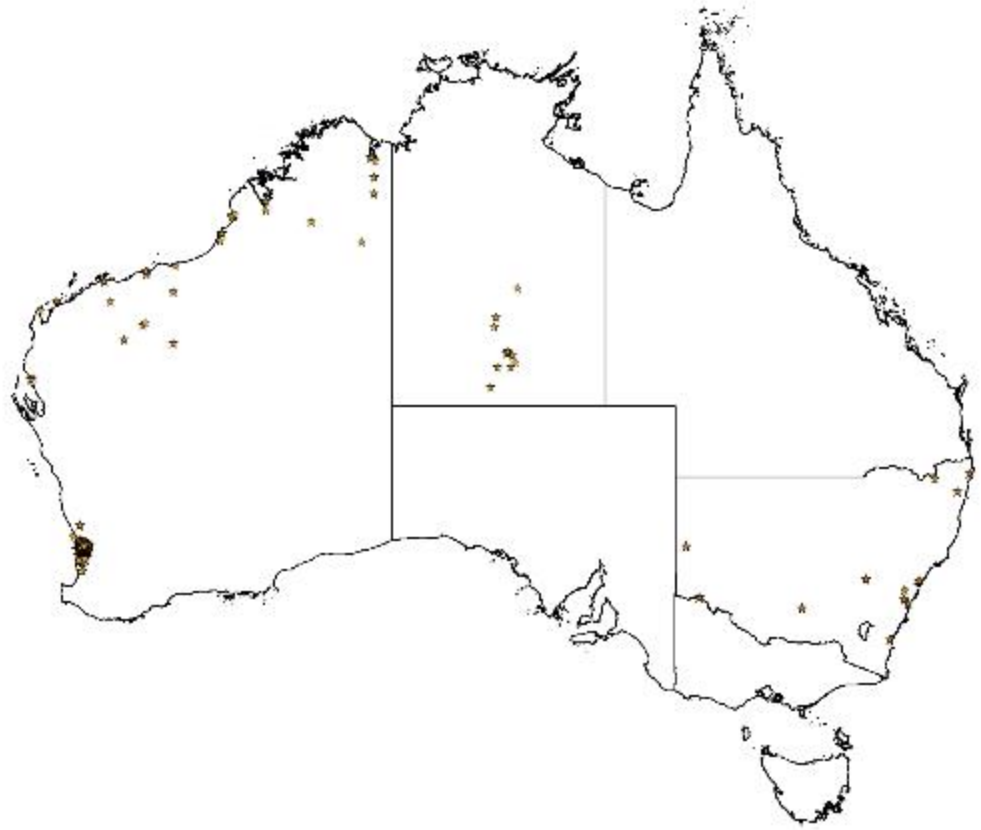
- Carry out targeted trapping for southern limit distribution
- Create or update Bioclim/ MaxEnt models

B. jarvisi
(Jarvis fruit fly)

Sultana S, Baumgartner JB, Dominiak BC, Royer JE, Beaumont LJ (2020) Impacts of climate change on high priority fruit fly species in Australia. PLoS ONE 15(2): e0213820

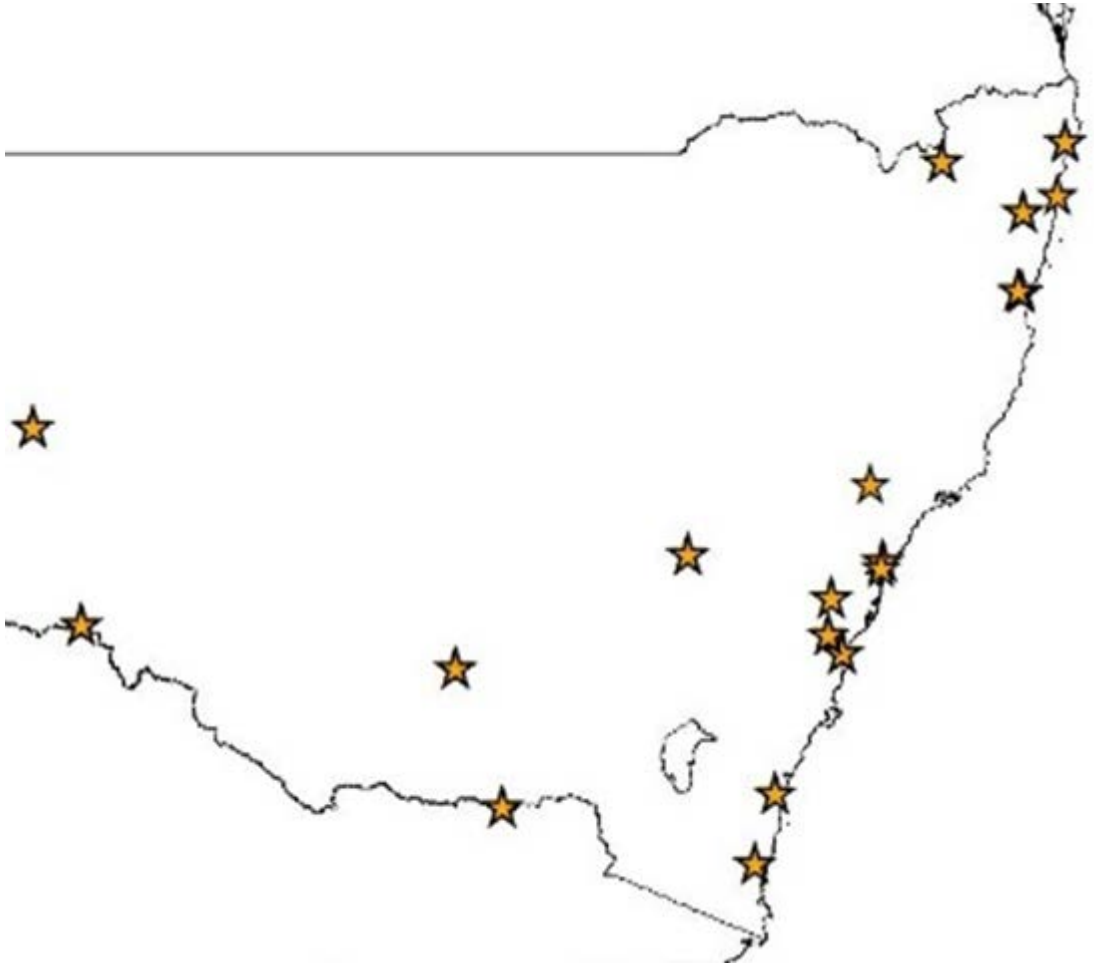
Trap site selection

- Fruit flies are distributed through
  - Natural dispersion
  - Human/ animal
    - Host fruit movement
- Trapping sites selected using previously recorded southern-limit as reference site

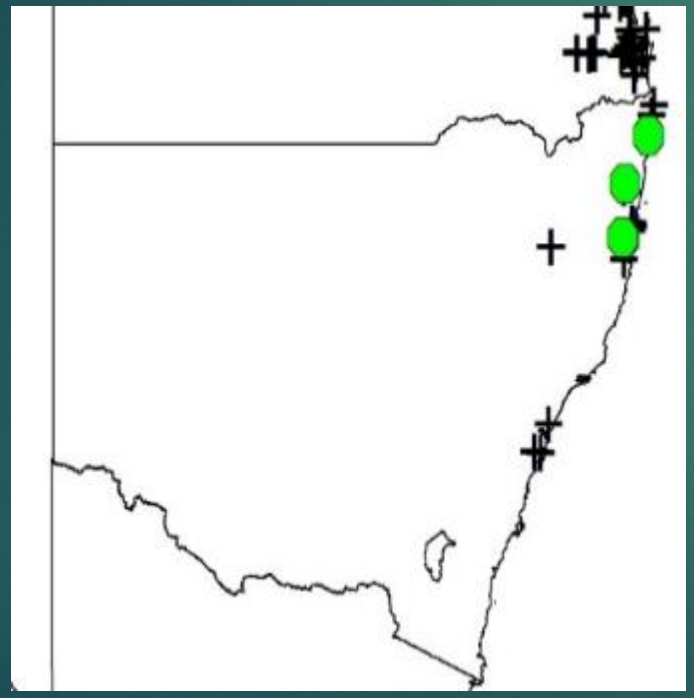


New South Wales

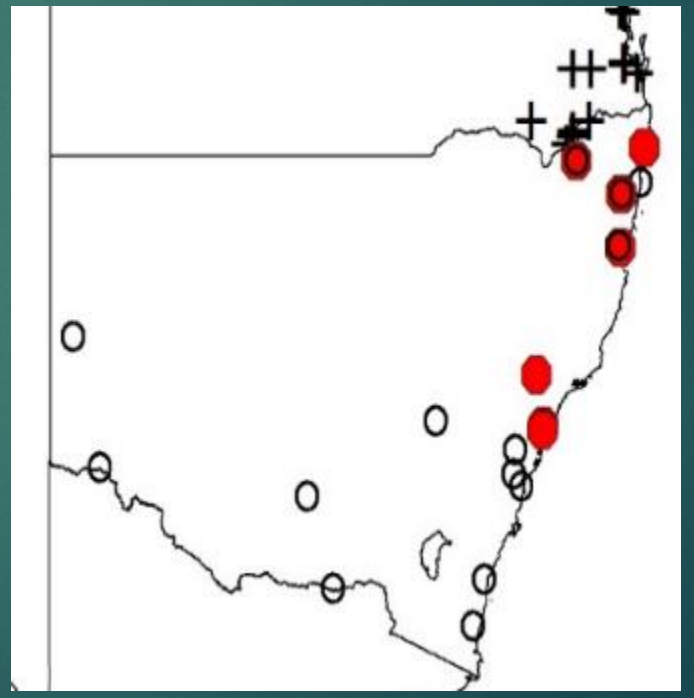
- North coast
- Central coast
- Sydney
- South coast
- Central-west
- South-west



- Southern limit
  B. neohumeralis



- Southern limit
  B. jarvisi



Aged lure analysis

Lure and toxicant analysis

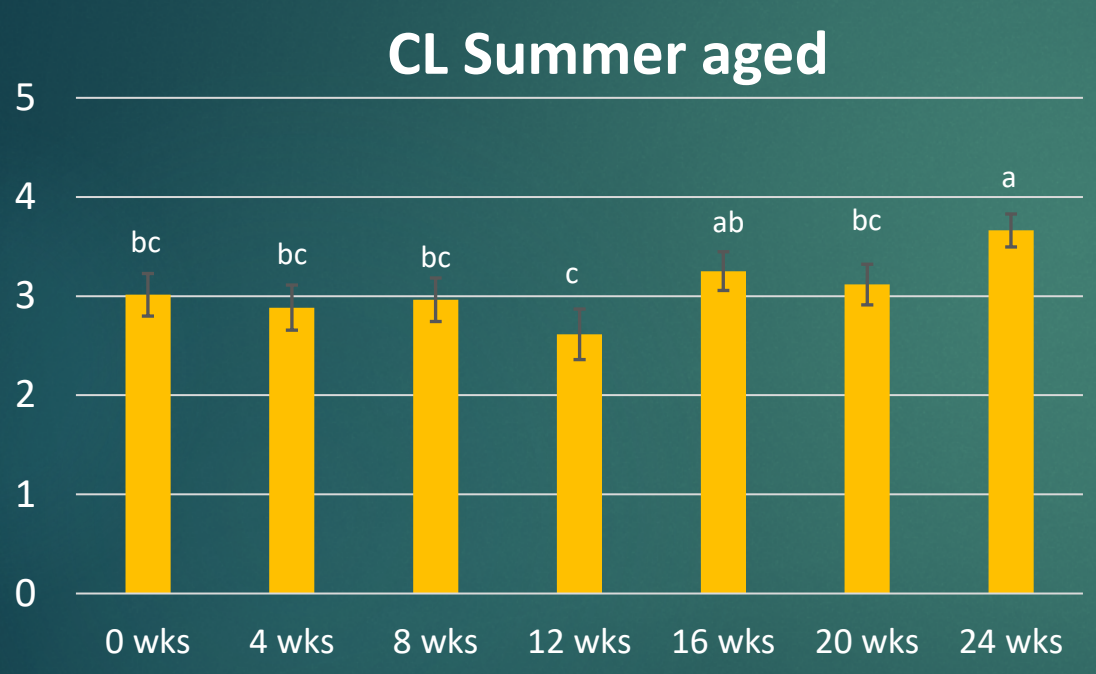
- 91 lure/toxicant from state collections
- 140 trial seasonally aged lures
- 56 CL, 56 ZN, 28 toxicant
- Total 231 lures

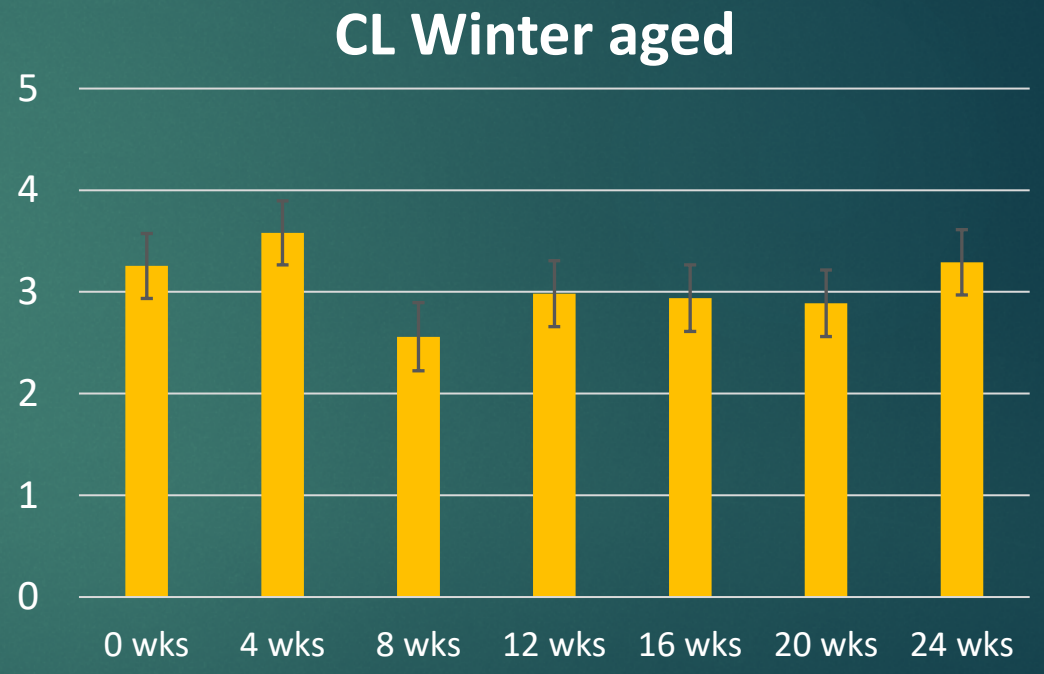
Aged lure efficacy trials

Two trials

- Cooler weather trial
- Hotter/humid weather trial
- Lures aged 0, 4, 8, 12, 16, 20, 24 weeks in traps
- Trials set up after all lure aged.

Hot weather and cool weather aged cue lure efficacy





Conclusions

- Chemical analysis will establish breakdown of lure and toxicant
- Cue lure and toxicant appear to be stable regardless of time of year on Atherton Tablelands
- Care must be taken due to low trial populations
- Research to compare lure devices is desirable
- Alternative toxicants should be evaluated

Fruit Fly Molecular Diagnostics

LAMP - aimed to develop and optimize laboratory protocols for new LAMP in-field diagnostic tools for key exotics (e.g. *B. trivialis*) and endemic species (e.g. *D. pornia, B. jarvisi*).

Metabarcoding - aimed to develop and validate tephritid fruit fly diagnostic molecular markers for simultaneous bulk DNA (metabarcoding) analysis of trap catches. Including, testing the specificity and sensitivity of fruit fly metabarcoding

Diagnostics – LAMP

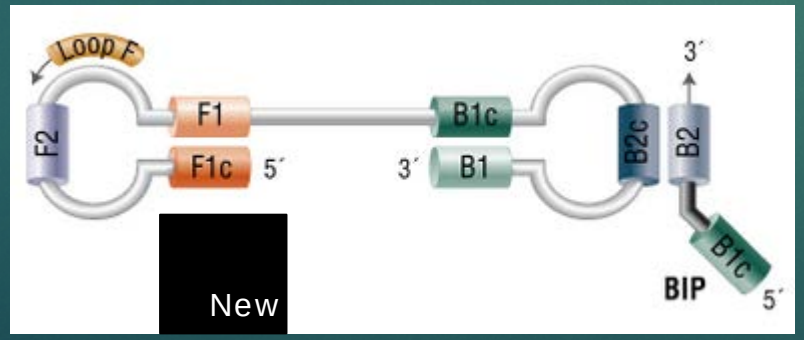
Nucleic Acids Research, 2000, Vol. 28, No. 12 e63

Loop-mediated isothermal amplification of DNA

Tsugunori Notomi[1,3,*], Hiroto Okayama[2], Harumi Masubuchi[1], Toshihiro Yonekawa[1], Keiko Watanabe[1], Nobuyuki Amino[3] and Tetsu Hase[1]

[1]Eiken Chemical Co. Ltd, 1381-3 Shimoishigami, Ohtawara, Tochigi 324-0036, Japan, [2]Department of Biochemistry and Molecular Biology, The University of Tokyo, Graduate School of Medicine, Bunkyo-ku, Tokyo 113-0033, Japan and [3]Department of Laboratory Medicine, Osaka University Medical School, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

Received February 1, 2000; Revised April 8, 2000; Accepted April 15, 2000



Diagnostics – LAMP

- Robust, rapid, portable diagnostic tests
- Can be conducted in the laboratory, or in the field
- Can use “crude” temperature based DNA extraction methods, retaining intact specimens for possible confirmation
- Complex primer design / optimisation of assays
- Target specific (Yes/No) diagnostics

Diagnostics – LAMP

Taxa	Group	Sample Type	DNA Extraction Method	TargetLocus	LAMP reagents	Amplification	Number of Primers	Visualisation
Ceratitis capitata	Mediterranean fruit fly	Adults, pupae, larvae, eggs	Spin column kit; QuickExtract; Chelex.	5.8 S rDNA (nuclear)	Custom made master mix	PCR Thermocycler	6	Fluorescence in tubes (SYBR Green)
Bactrocera dorsalis group, *B. latifrons*, & *Zeugodacus cucurbitae*	Fruit flies	Larvae	Alkaline lysis solution (KOH)	5′-COI (mitochondrial)	Commercial master mix	Real-time fluorometer (Genie II), & Real-time qPCR thermocycler	6	Fluorescence measured in real-time fluorometer (SYBR Green)
Dacus ciliatus	Ethiopian fruit fly	Adults, pupae, larvae, eggs	Distilled water	5′-COI (mitochondrial)	Custom made master mix	PCR Thermocycler	4	Fluorescence in tubes (Calcein)
Drosophila suzukii	Spotted winged drosophila	Adults	Spin column kit	Ds10_00012111 (nuclear)	Custom made master mix	PCR Thermocycler	4	Fluorescence in tubes (SYBR Green)
Anopholes gambiae & *A. arabiensis*	Mosquitoes	Adults	Spin column kit; NaCl buffer.	IGS 28 S rDNA (nuclear)	Commercial master mix	PCR Thermocycler	4	Fluorescence in tubes (Calcein)

Blacket *et al.* 2020
Scientific Reports

Diagnostics – LAMP

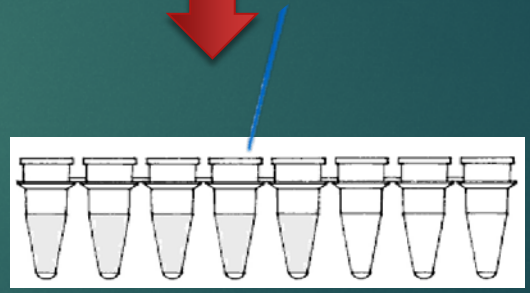
1.

Samples: Add samples (adult, larvae, pupae or eggs) to DNA QuickExtract buffer with a single use pin

2.

DNA Extraction: Incubate in Genie III 65°C (6 mins), then 98°C (2 mins)

3.



Master Mix: Add 1 µL of DNA to LAMP assay 6 primers + reaction mix (DR001)

4.

LAMP reaction:
Amplification 65°C (25 mins)
Annealing 98°C to 73°C (10 mins)
Total run time 35 mins

Diagnostics – LAMP

Field Testing: Victoria

Fruit fly larva

B. tryoni

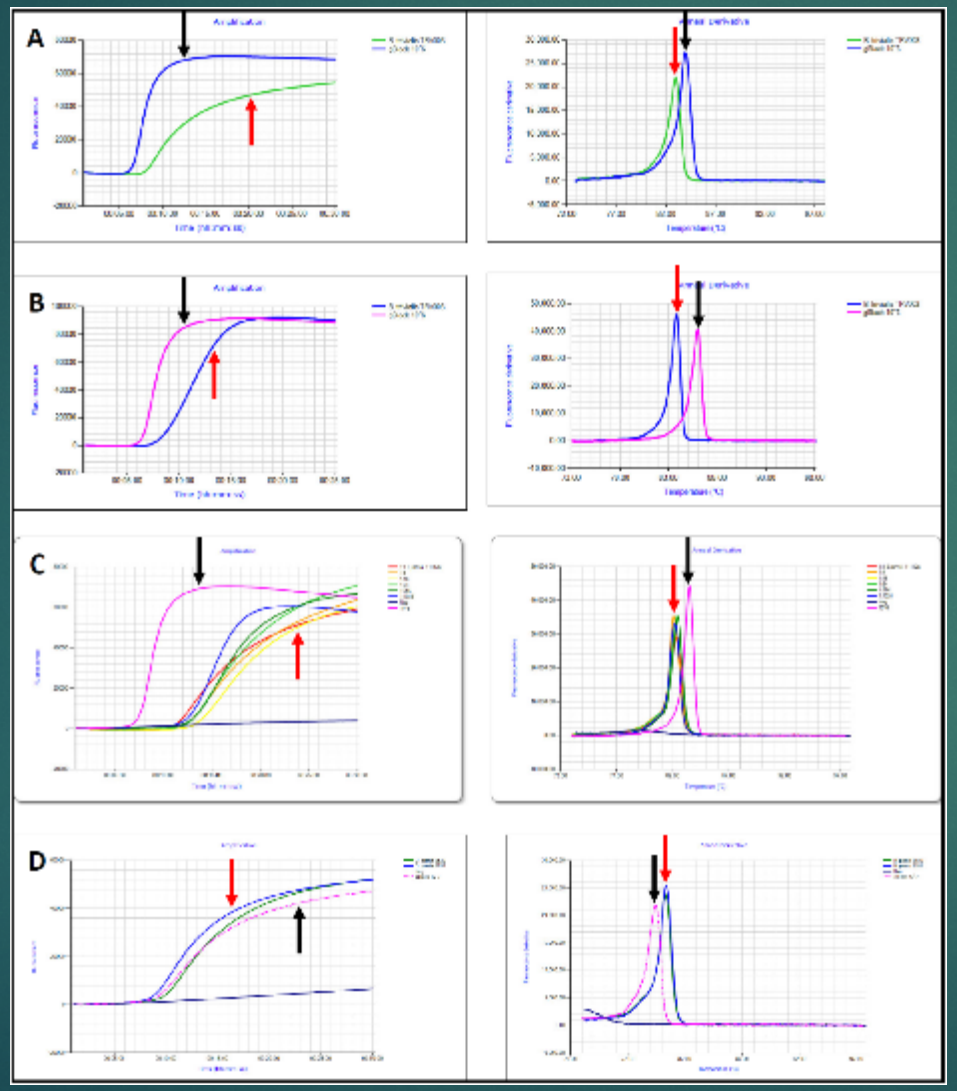
QuickExtract DNA

LAMP assay

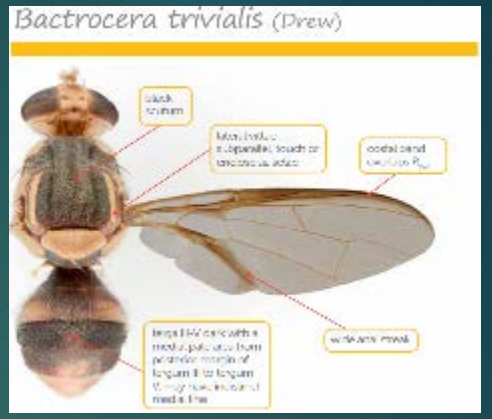
Immediate actions: positive samples (treatment)
negative samples (release produce)

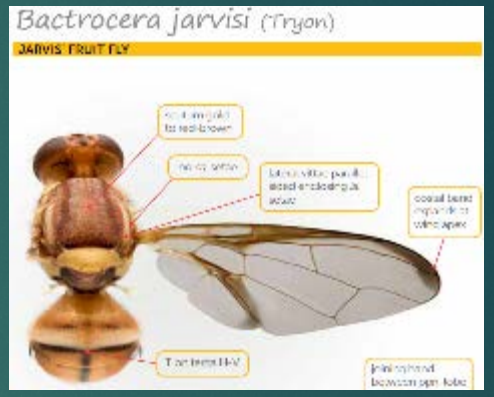
Blacket *et al.* 2020
Scientific Reports

Diagnostics – LAMP



New LAMP Assays





PHA 2018

Diagnostics – LAMP

LAMP assays for detection of the New Guinea fruit fly *Bactrocera trivialis* (Drew) (Diptera: Tephritidae)

Melissa L. Starkie (1)*, Elizabeth V. Fowler (1), Xiaocheng Zhu (2), Arati Agarwal (3), Lea Rako (3), Isarena C. Schneider (4), Mark K. Schutze (1), Jane E. Royer (1), David Gopurenko (2), Peter Gillespie (2), Mark J. Blacket (3)

(1) Biosecurity Queensland, Department of Agriculture and Fisheries, Brisbane, QLD,

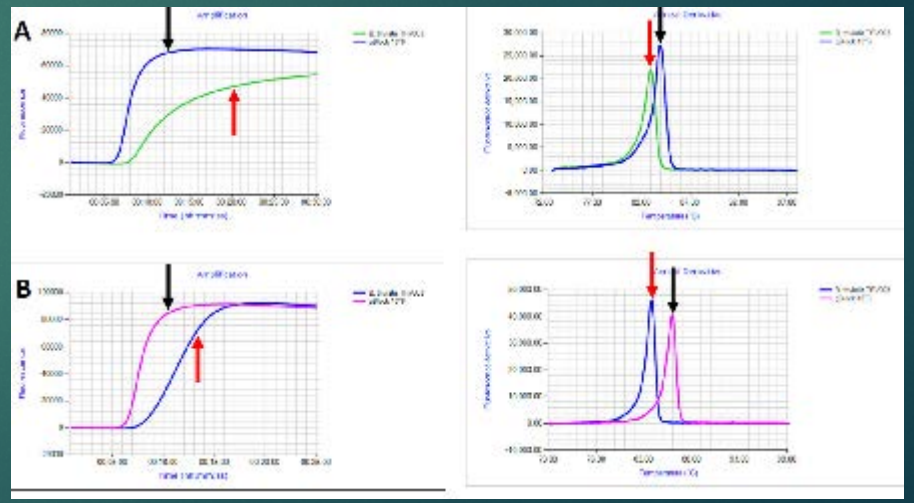
(2) New South Wales Department of Primary Industries, Orange NSW

(3) Agriculture Victoria, Department of Jobs, Precincts and Regions, Bundoora, VIC

(4) Department of Agriculture, Water and the Environment, Cairns, QLD

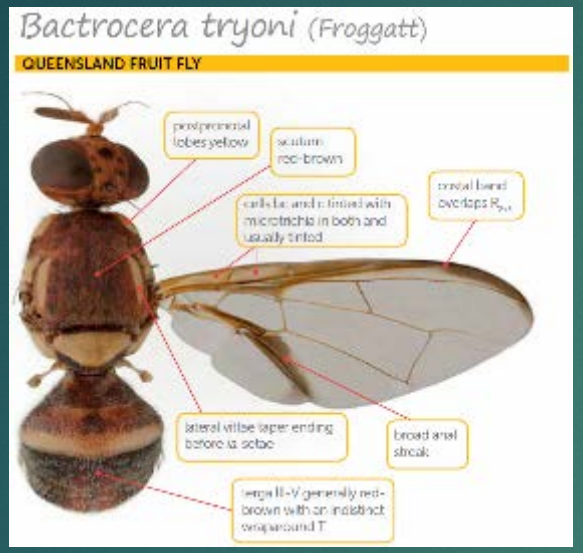
Abstract

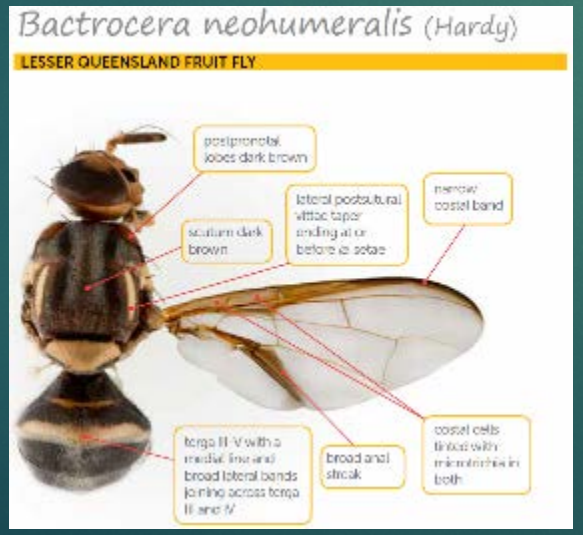
The cue-lure-responding New Guinea fruit fly, *Bactrocera trivialis*, poses a biosecurity risk to neighbouring countries, e.g., Australia. In trapping programs, lure caught flies are usually morphologically discriminated from non-target species; however, DNA barcoding can be used to confirm similar species where morphology is inconclusive, e.g., *Bactrocera breviaculeus* and *B. rufofuscula*. This can take days – and a laboratory – to resolve. A quicker, simpler, molecular diagnostic assay would facilitate a more rapid detection and potential incursion response. We developed LAMP assays targeting cytochrome c oxidase subunit I (COI) and Eukaryotic Translation Initiation Factor 3 Subunit L (EIF3L); both assays detected B. trivialis within 25 min. The BtrivCOI and BtrivEIF3L assay anneal derivatives were 82.7 ± 0.8°C and 83.3 ± 1.3°C, respectively, detecting down to 1×101 copies/µL and 1×103 copies/µL, respectively. Each assay amplified some non-targets from our test panel; however notably, BtrivCOI eliminated all morphologically similar non-targets, and combined, the assays eliminated all non-targets. Double-stranded DNA gBlocks were developed as positive controls; anneal derivatives for the COI and EIF3L gBlocks were 84.1 ± 0.7°C and 85.8 ± 0.2°C, respectively. We recommend the BtrivCOI assay for confirmation of suspect cue-lure-trapped B. trivialis, with BtrivEIF3L used for secondary confirmation when required.



Starkie *et al.* 2022
Scientific Reports (Submitted)

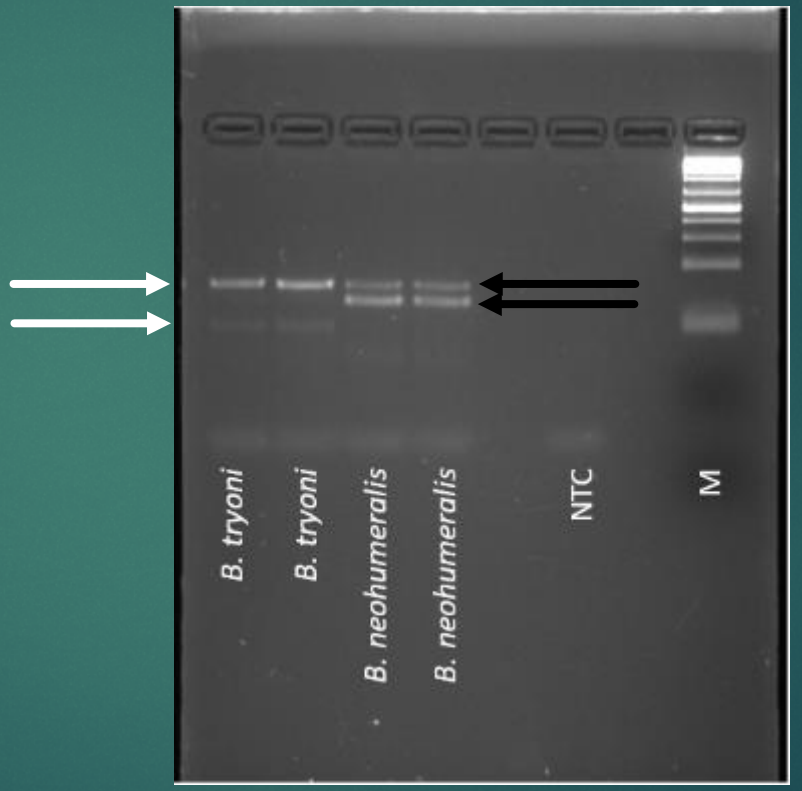
Diagnostics – SNP Assay





PHA 2018

Diagnostic SNP's detected through PCR

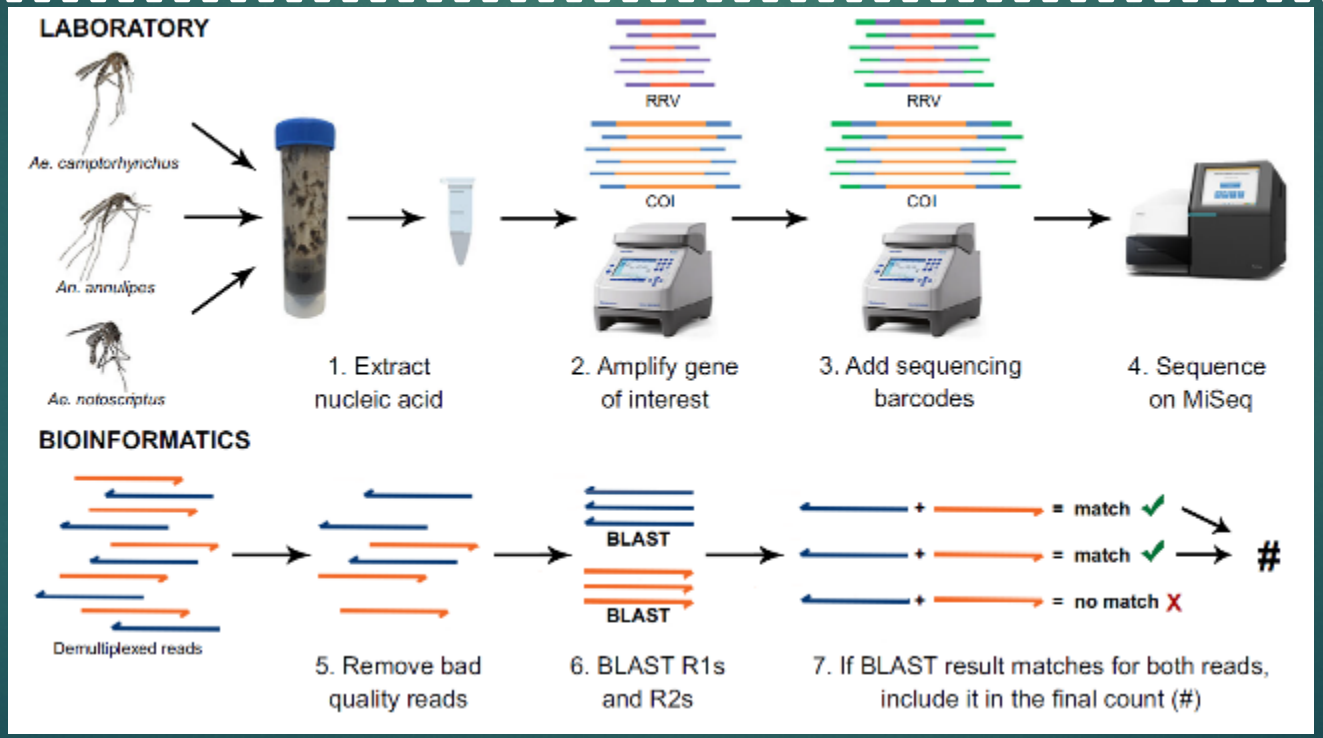


New SNP Assay

Diagnostics – Metabarcoding

Traditional Microscopic Identification →

Morphology

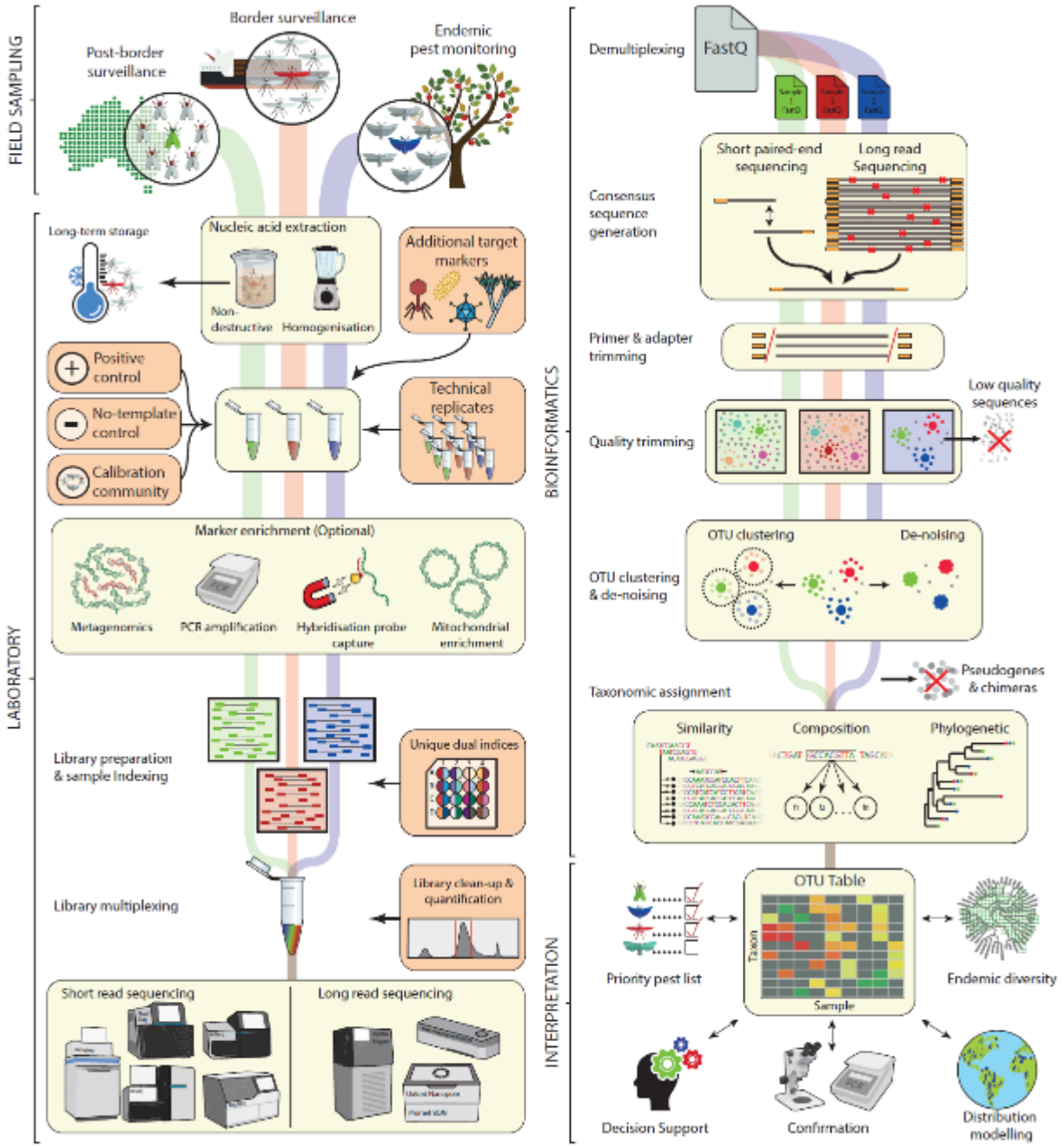


Metabarcoding

Lab based

Non-targeted diagnostics

Batovska *et al.* 2018
Molecular Ecology Resources

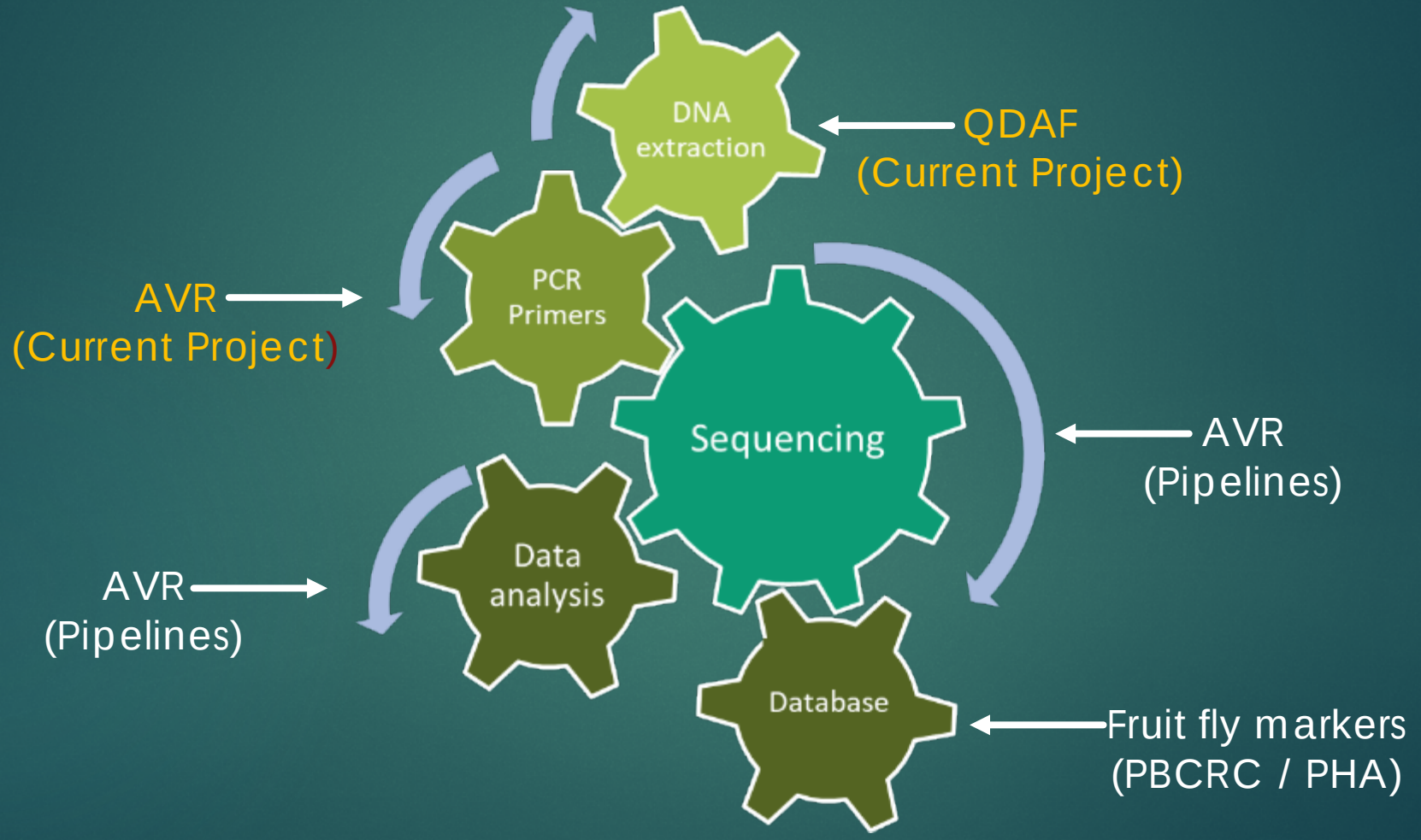


Diagnostics – Metabarcoding

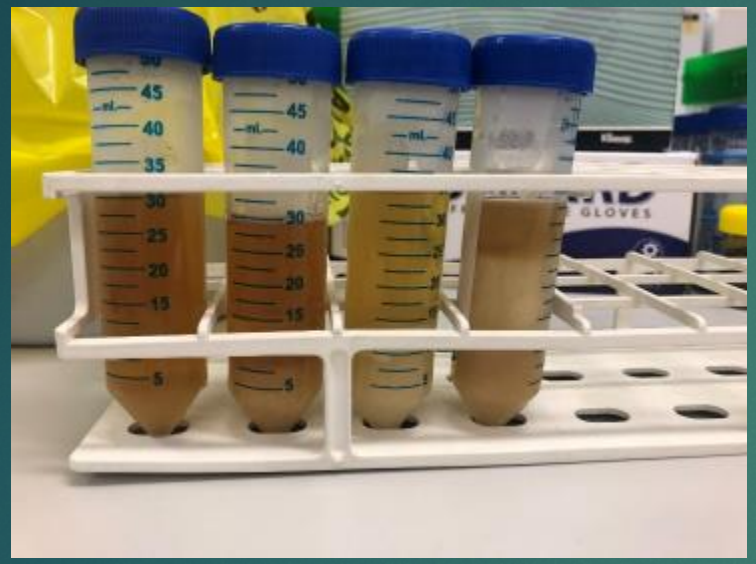
PIPER ET AL. 2019

GIGASCIENCE

Diagnostics – Metabarcoding



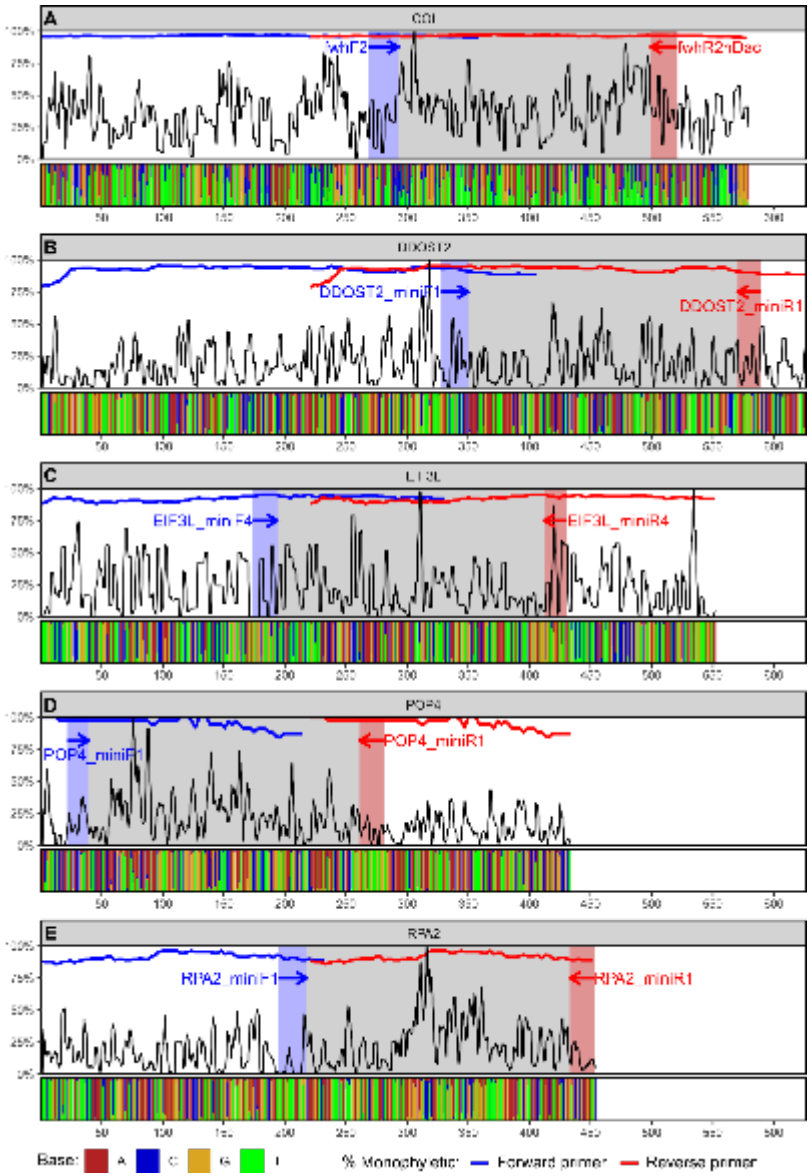
Diagnostics – Metabarcoding

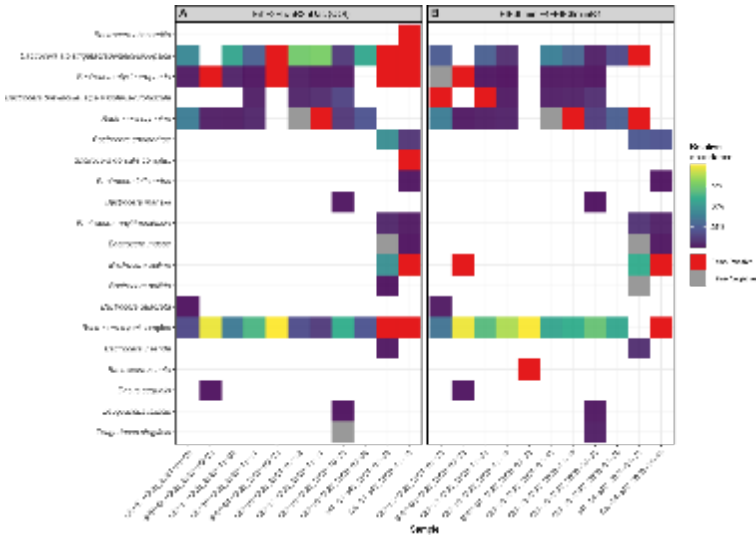


- Bulk DNA extraction method optimised for processing 1000's of flies
- Non-destructive, retains morphology
- Tested through qPCR
- Optimised for metabarcoding

Diagnostics – Metabarcoding

- Initial metabarcoding results COI & EIF3L
- Additional traps and sensitivity testing underway
- Laboratory training to be completed in 2022





Diagnostics – SOP's

- New protocols tested in multiple laboratories

	SOP	SOP Developed	SOP Validated
1	Non-destructive extraction protocol (Appendix A)	QDAF	NSW DPI, AgVic
2	Real-time qPCR detection of Jarvis' fruit fly, *Bactrocera jarvisi* (Appendix B)	QDAF	NSW DPI, AgVic
3	Real-time qPCR detection of cucumber fruit fly, *Zeugodacus cucumis* (Appendix C)	QDAF	NSW DPI
4	Real-time qPCR detection of Queensland fruit fly, *Bactrocera tryoni* (Appendix D)	QDAF	NSW DPI, AgVic
5	LAMP detection of Queensland fruit fly, *Bactrocera tryoni* (Appendix E)	QDAF	NSW DPI, AgVic
6	LAMP detection of New Guinea fruit fly, *Bactrocera trivialis* (Appendix F)	QDAF	NSW DPI, AgVic
7	LAMP detection of Jarvis' fruit fly *Bactrocera jarvisi* (Appendix G)	AgVic	NSW DPI, QDAF
8	LAMP detection of Island fruit fly *Dirioxa pornia* (Appendix H)	AgVic	QDAF

Diagnostics – Trap Design

- Field testing (Qld sites) compared with existing Steiner, Paton and Lynfield traps.
- Also, the effect of temperature, humidity, time on DNA quality is being investigated.

Enhanced Steiner trap (left), Paton trap version 1 (middle), Paton trap version 2 (right), showing new elements to reduce water entry.

“Phenology, demography and distribution of Australia’s fruit flies” is funded through the Strengthening Australia’s Fruit Fly System Research Program of the Department of Agriculture Water & Environment, with matched funds from State and Territory governments