

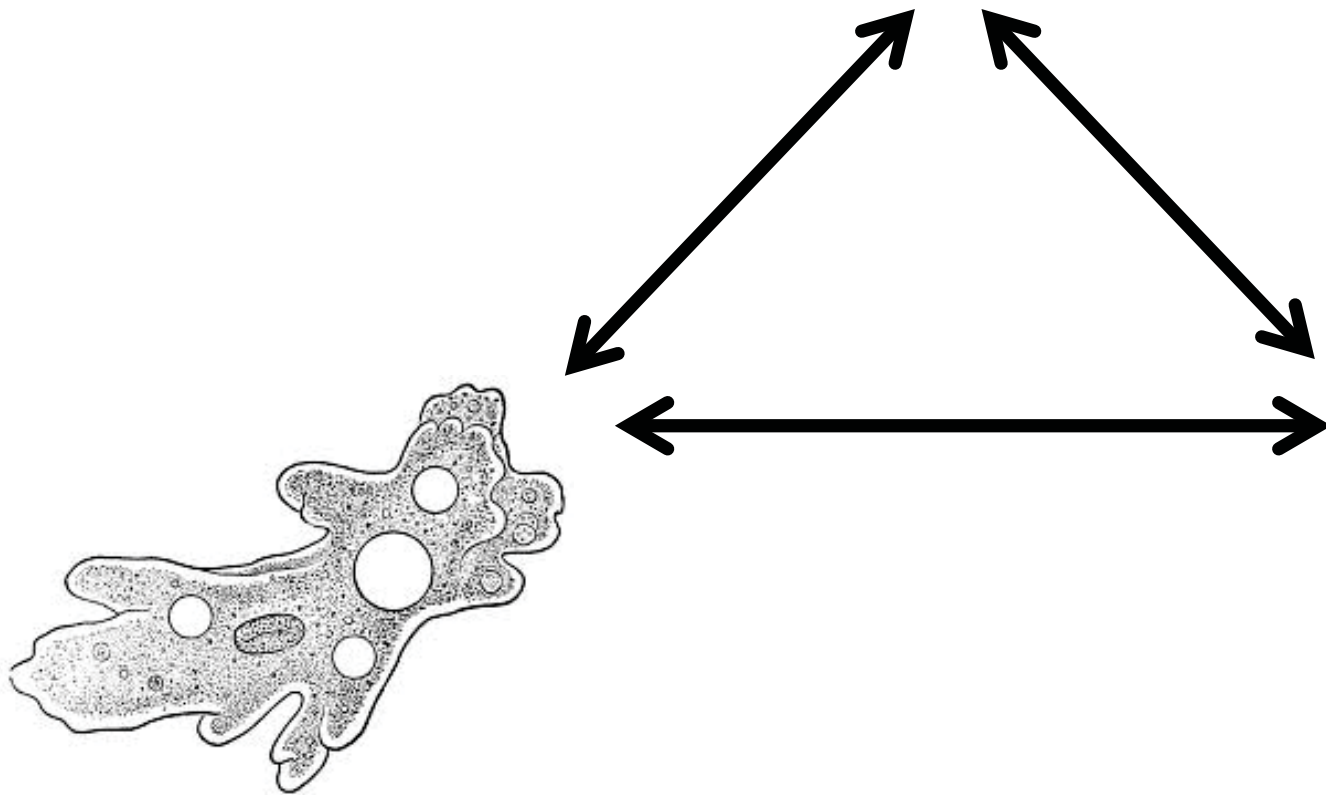


# An update on CSIRO research into AGD in Tasmania

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FOOD FUTURES FLAGSHIP  
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# Environment – water sampling, traditionally labour intensive and replication difficult

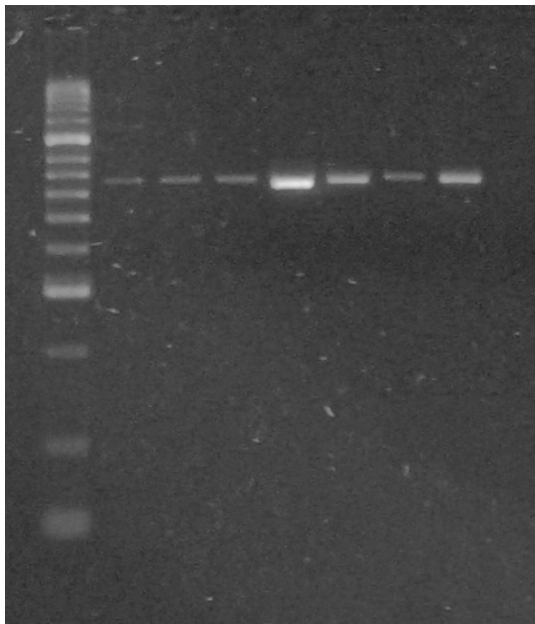




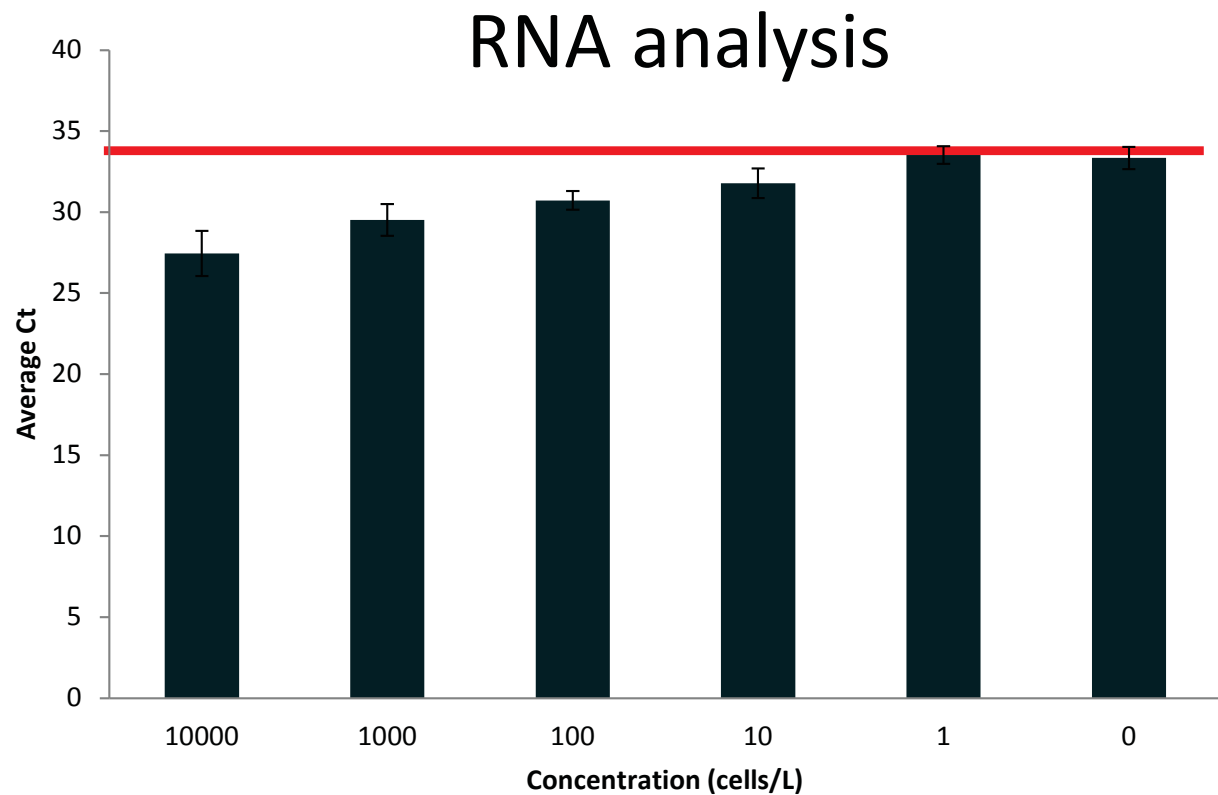
# Semi-automatic sampling in situ



# Results – Semi-automated water sampling



DNA Analysis



# 2<sup>nd</sup> Generation sampler



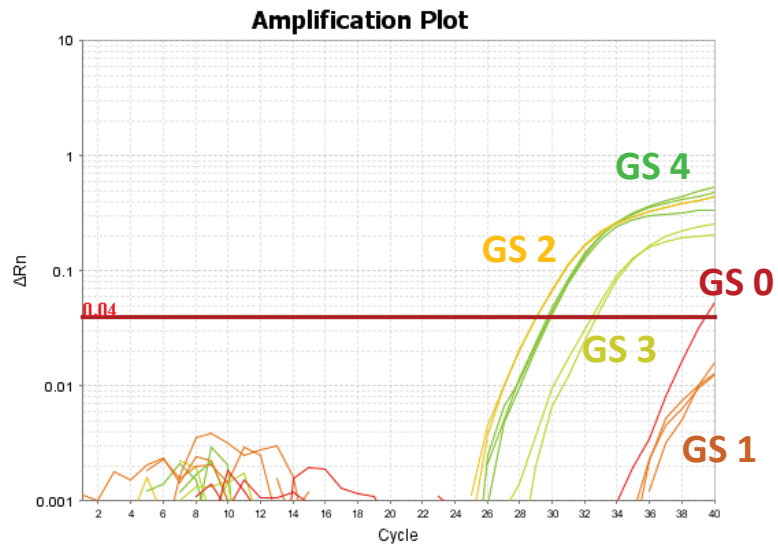
- Computer controlled – set and forget
- Capable of taking and archiving 24 samples
- Pumps water across filter and then ‘floods’ with appropriate buffer
- Will be deployed 2<sup>nd</sup> ½ of 2014

# Non-destructive sampling of gills - swabs

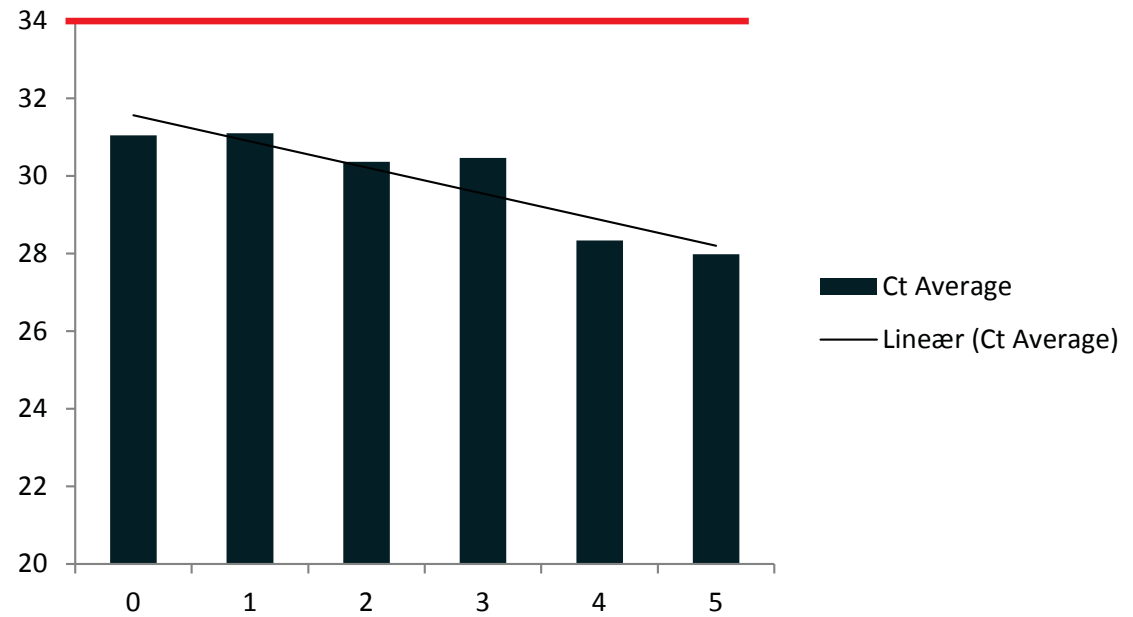


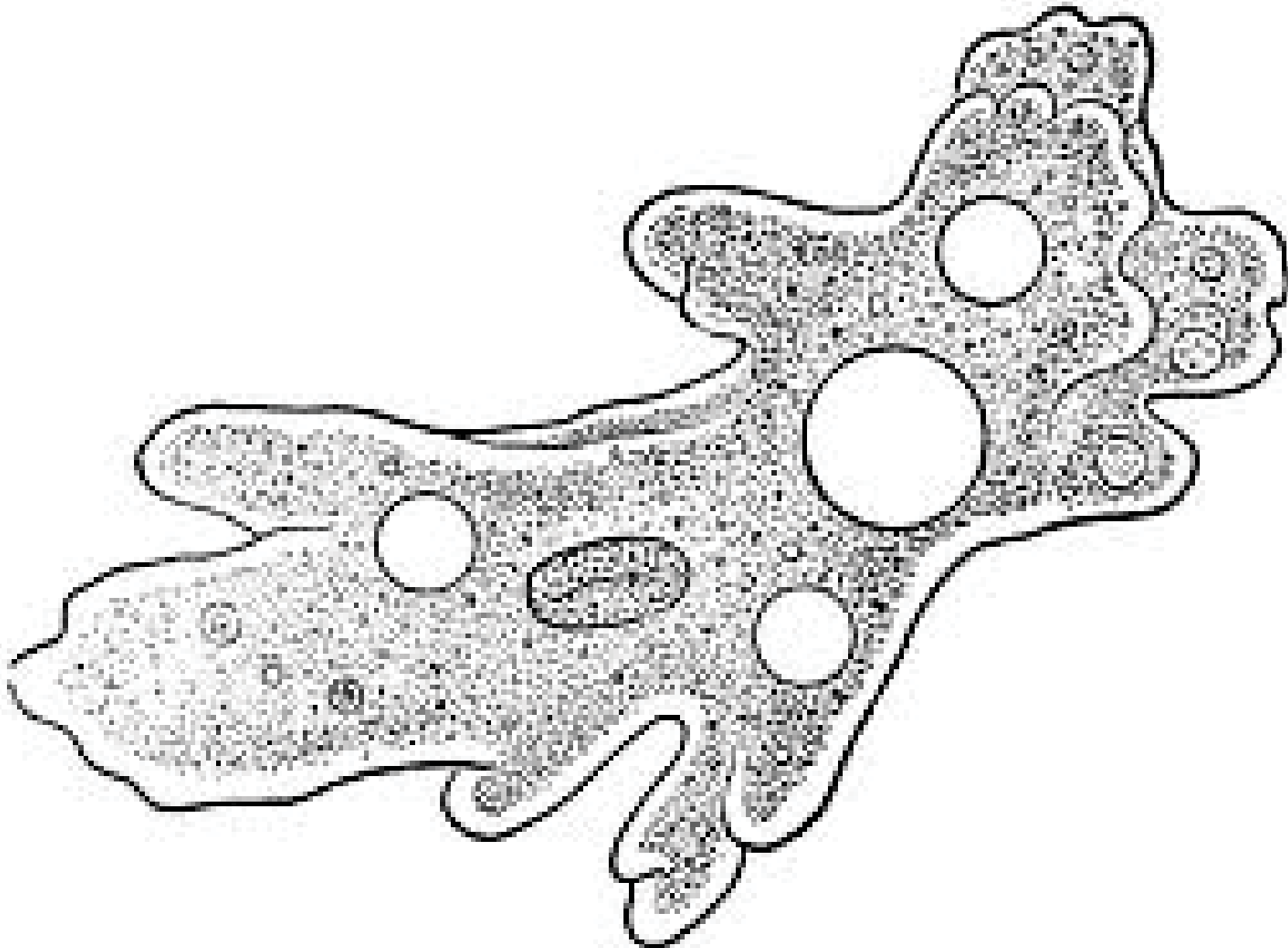


# RNA vs DNA results

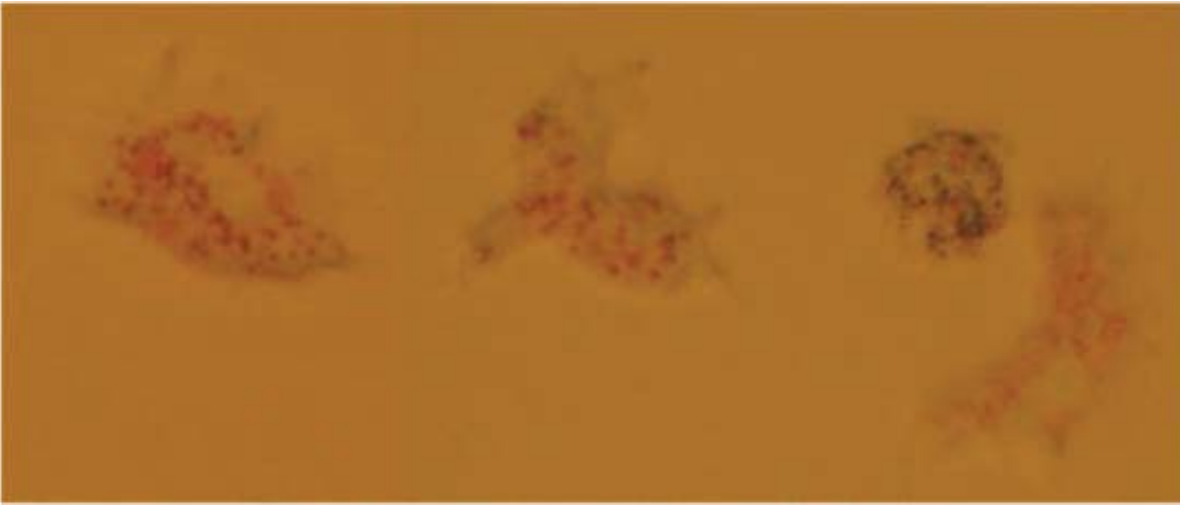


Average Cycle Threshold Value per GS Score





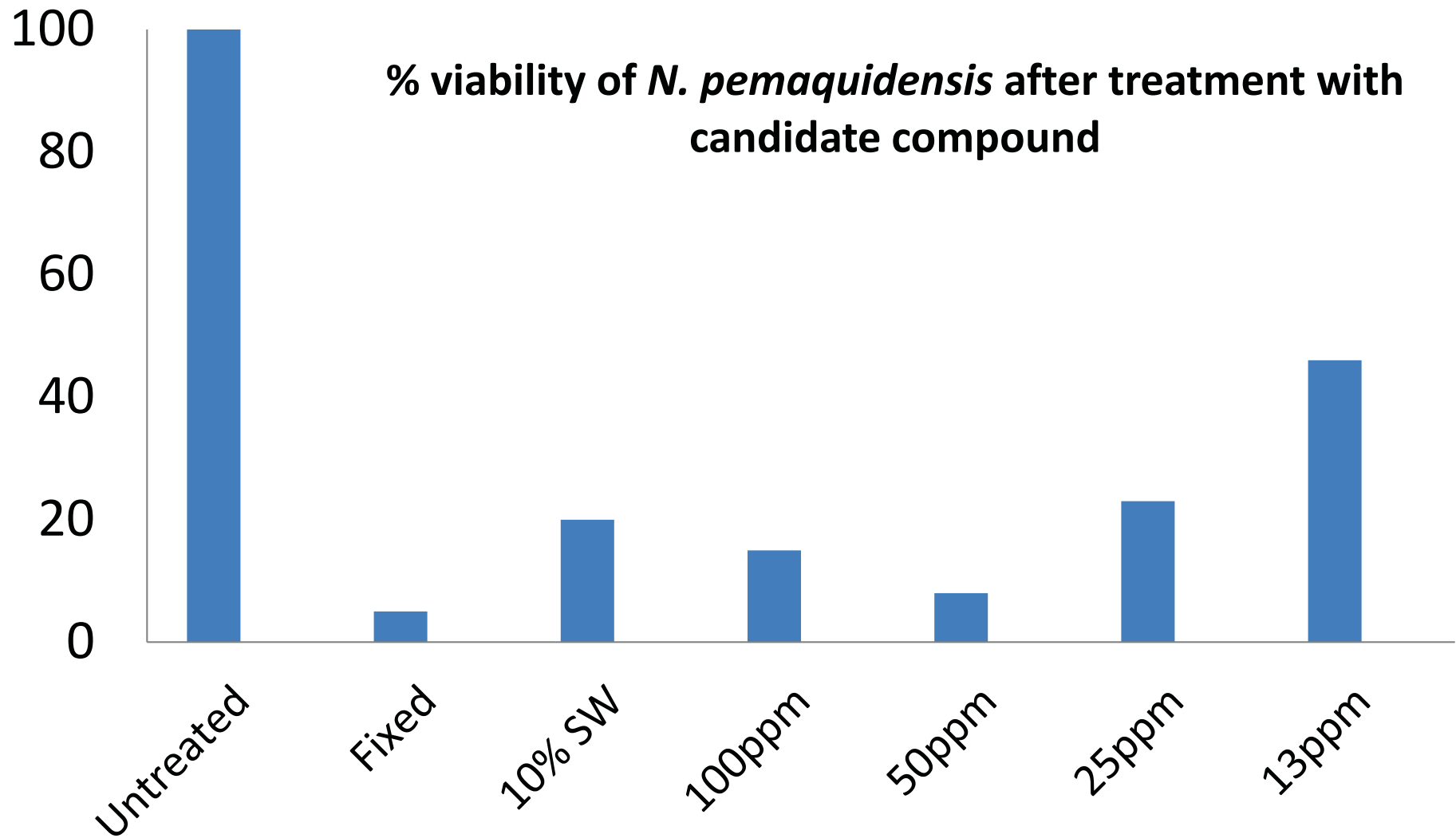
# Development of Amoebae Bioassay



- Assay based on the observation that *P. perurans* actively 'takes up' Neutral red dye
- Apply treatments to observe 'uptake'
- Requires appropriate controls (+ve and -ve)

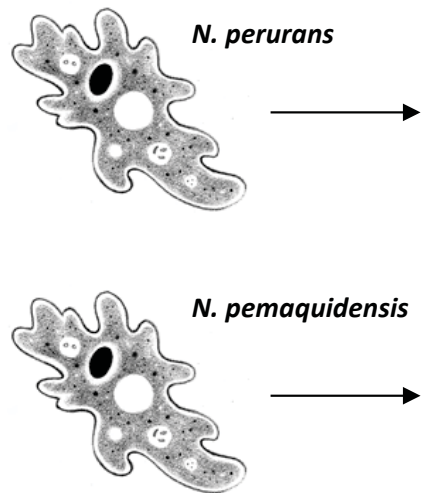


# Indicative results



# Transcriptomic analysis

Compare genetic information of amoeba *P. Perurans* (infective) and closely related species *P. Pemaquidensis* (non-infective) to identify differences.



Seq <sub>01</sub> : ACGGTAGGCTAGACTAGATATTAACG	
Seq <sub>02</sub> : CCTGAGTACCTGGACTAGATAAC	
Seq <sub>03</sub> : GATGCGGTTACGTACGATCCATGGA	
Seq <sub>04</sub> : CATTATTATATATACGCGCGCGA	
Seq <sub>05</sub> : TTTCGATAGGGGATATATTAACGCCG	
Seq <sub>06</sub> : GTAGGTAGGTGGAGGCCCGCAGACCG	
Seq <sub>07</sub> : GATAGACTCGCGCCGATATATAG	
Seq <sub>08</sub> : ATATATTTCCCTAGATCGAGAGATAC	
Seq <sub>09</sub> : GATAGGTTAATTAATTTCCCTATAT	
Seq <sub>10</sub> : TGGATTGGATAGCGCGATAGATC	
Seq <sub>11</sub> : AAAAGTCGATAAGGCTAGAGCTAG	
Seq <sub>12</sub> : GGATATAGATATATCTAGATATC	
Seq <sub>13</sub> : CGATATAGCCCTCTAGAGATACTTT	
Seq <sub>14</sub> : GATACCCGCGATATATCAT	
Seq <sub>15</sub> : TAGATCCCCGAGATAGAGACT	
Seq <sub>16</sub> : CACCATAGAAGACTGATCGAGATAG	

Compare the two datasets to identify:

1. Transcripts (i.e., **functionality**) present in one dataset that are “missing” in the other dataset?
2. Of those transcripts that are similar, how similar are they?

# Raw Read Support

ACGTTA	TTAACG	ACCGCA
TTAACG	GGCACG	TATACG
TACGTA	AACGTA	CCGGTA
TACAGT	TACAGT	TACAGT
CATAGT	CATAGT	CATAGT
AGTCAT	AGTCAT	AGTCAT
GTACAT	GTACAT	GTACAT
CATGTA	CATGTA	CATGTA
ACGTTA	ACGTTA	ACGTTA
TTAACG	TTAACG	TTAACG
TACGTA	TACGTA	TACGTA
TACAGT	TACAGT	TACAGT
CATAGT	CATAGT	CATAGT
AGTCAT	AGTCAT	AGTCAT
GTACAT	GTACAT	GTACAT
CATGTA	CATGTA	CATGTA



BLAST



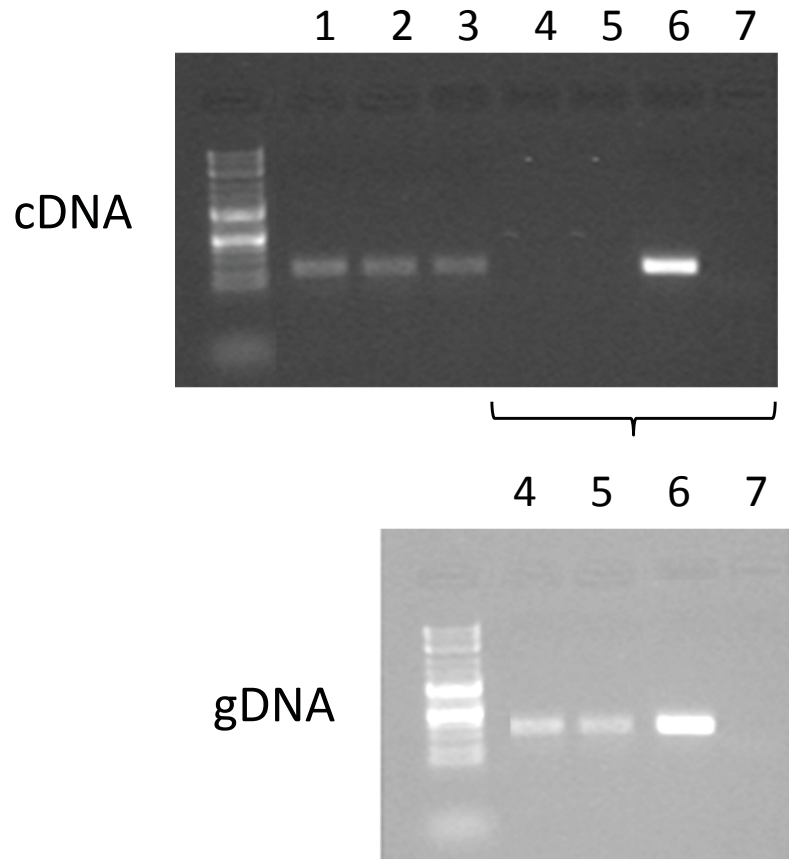
ACGGTAGGCTAGACTAGATATTAACG
CCTGAGTACCTGGACTAGATAC
GATGCGGTACGTACGATCCATGGA
CATTTATTATATATACGCGCGGA
TTTCGATAGGGGATATATTAACGCCG
GTAGGTAGGTGGAGGCCCGCAGACGC
GATAGACTCGCGCCGATATATAG
ATATATTTCTAGATCGAGAGATAC
CCTGAGTACCTGGACTAGATAC
GTAGGTAGGTGGAGGCCCGCAGACGC
ACGGTAGGCTAGACTAGATATTAACG
GTAGGTAGGTGGAGGCCCGCAGACGC

ACGTTA	
AGTCAT	AGTCAT
CATGTA	CATGTA
<b>GTAGGTACGTGGAGGCCCGCAGACGC</b>	
CATGTA	AGTCAT
TCAACG	ACGTTA
TACGTA	



# We can turn off genes in *P. perurans*

GSP for 'Target'



time	Average Ct	
	Target	18S
Untreated	28.2	22.5
24hr	28.7	22.9
48hr	29.8	23.6
72hr	32	23.9
1week	31.7	24.9
NTC	32.5	34.4

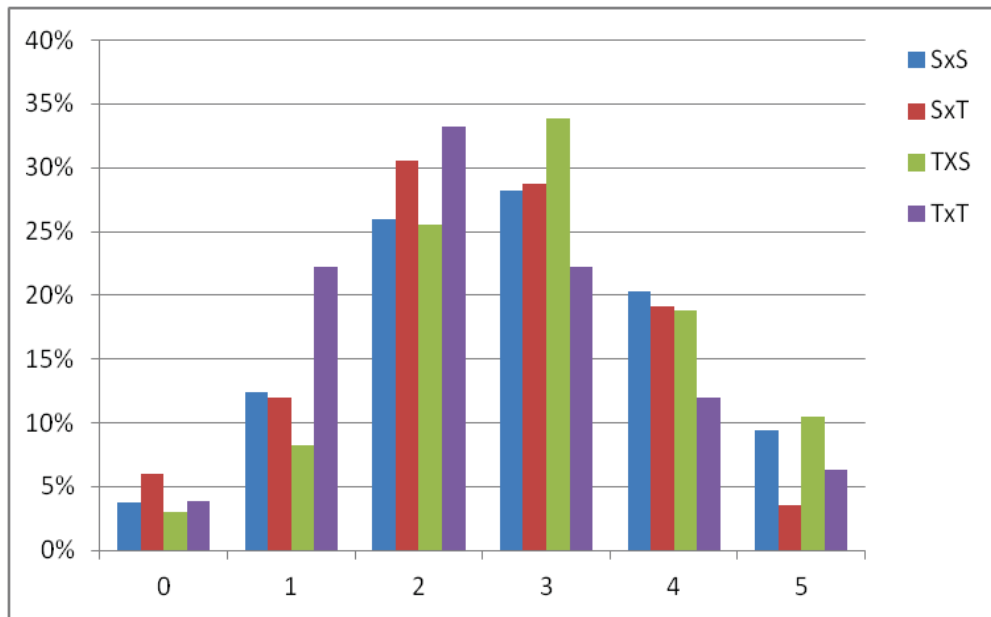
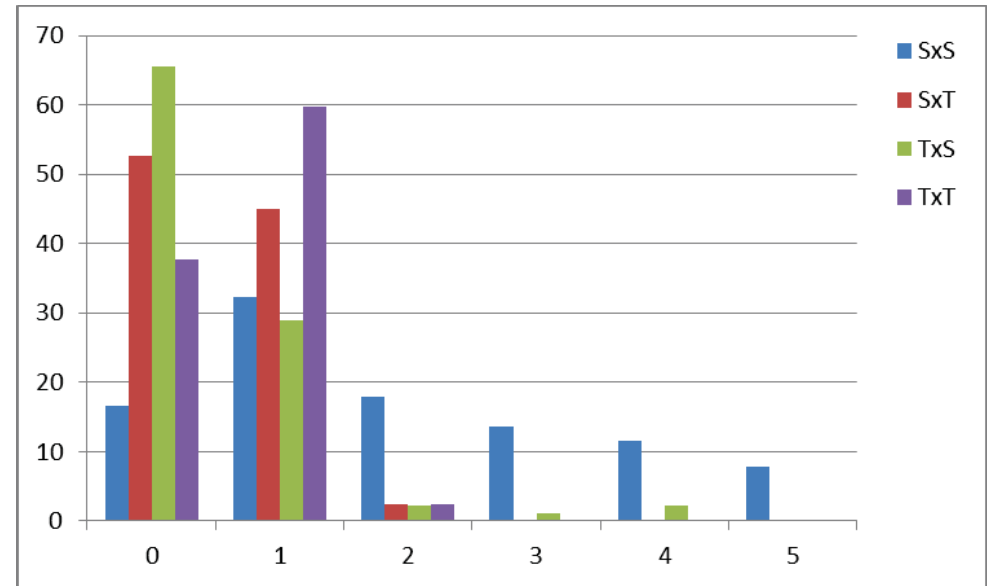
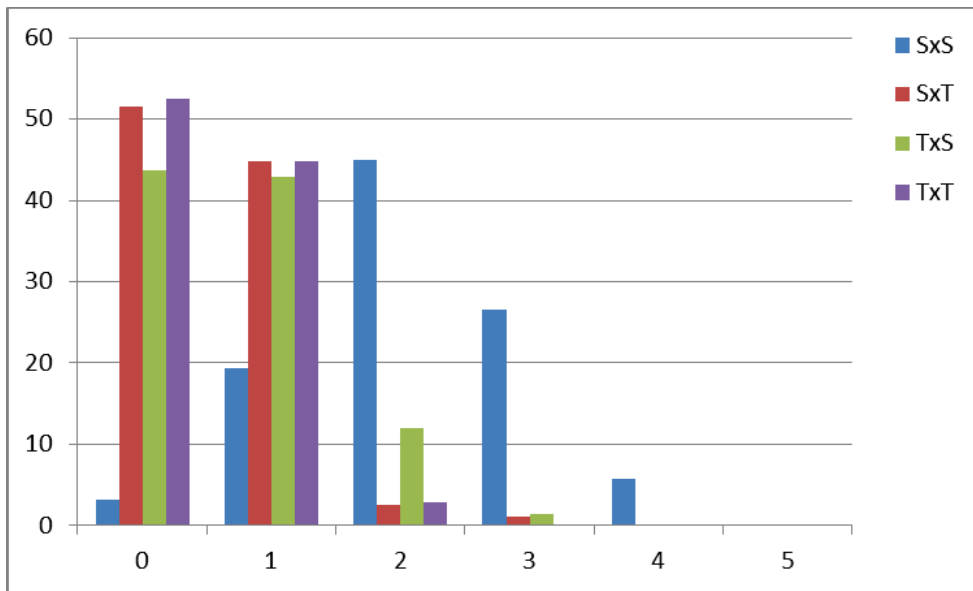




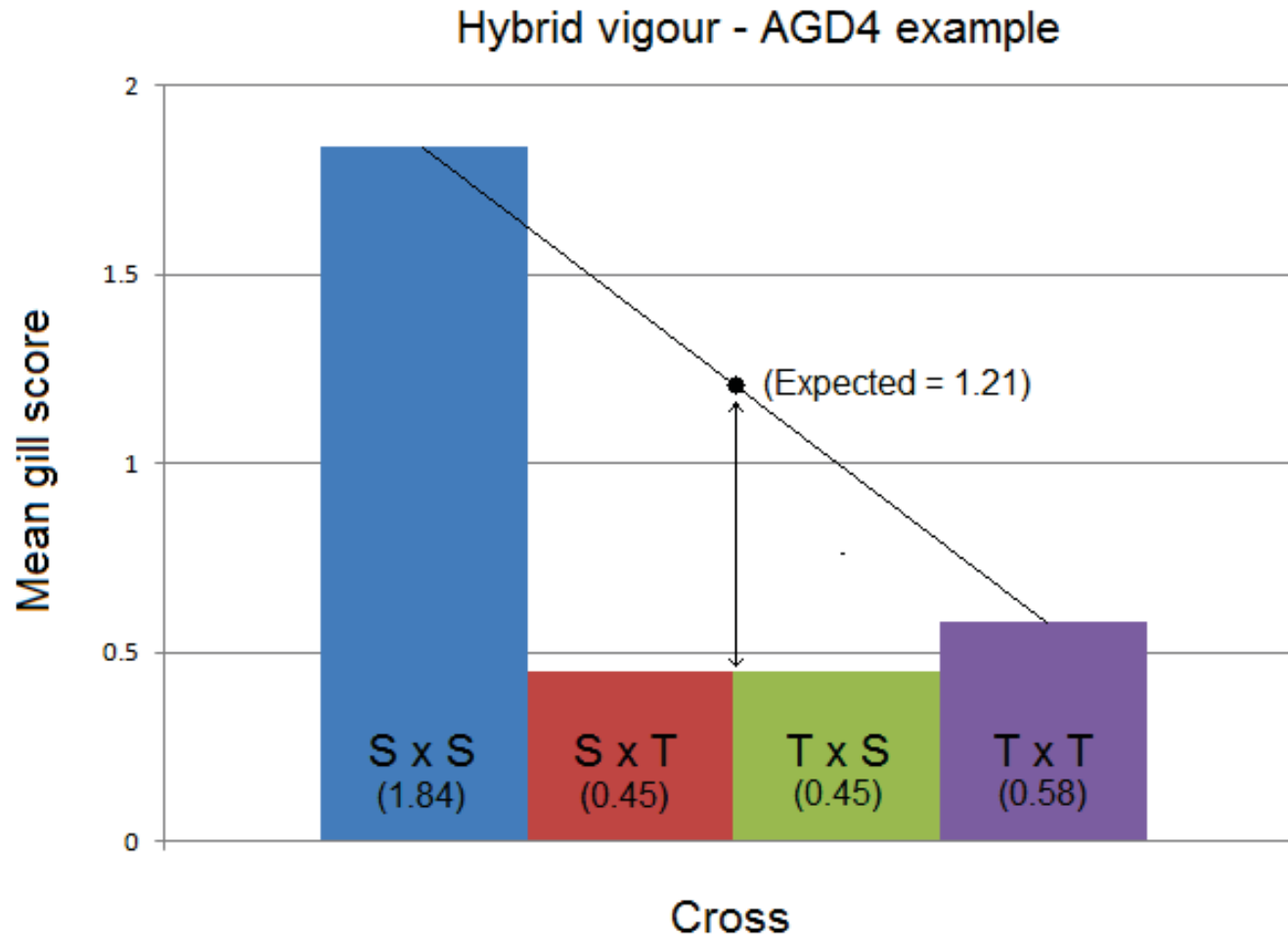
# Hybrids – can we unlock the ‘key’ to resistance







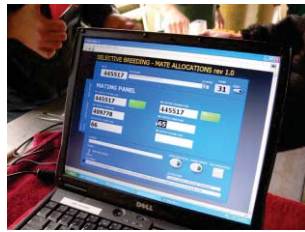
# Hybrid vigour



- 63% hybrid vigour observed at AGD4
- “Best parent” performance

# Breeding for AGD Resistance

Broodstock never go to sea



genetic evaluation  
and selection



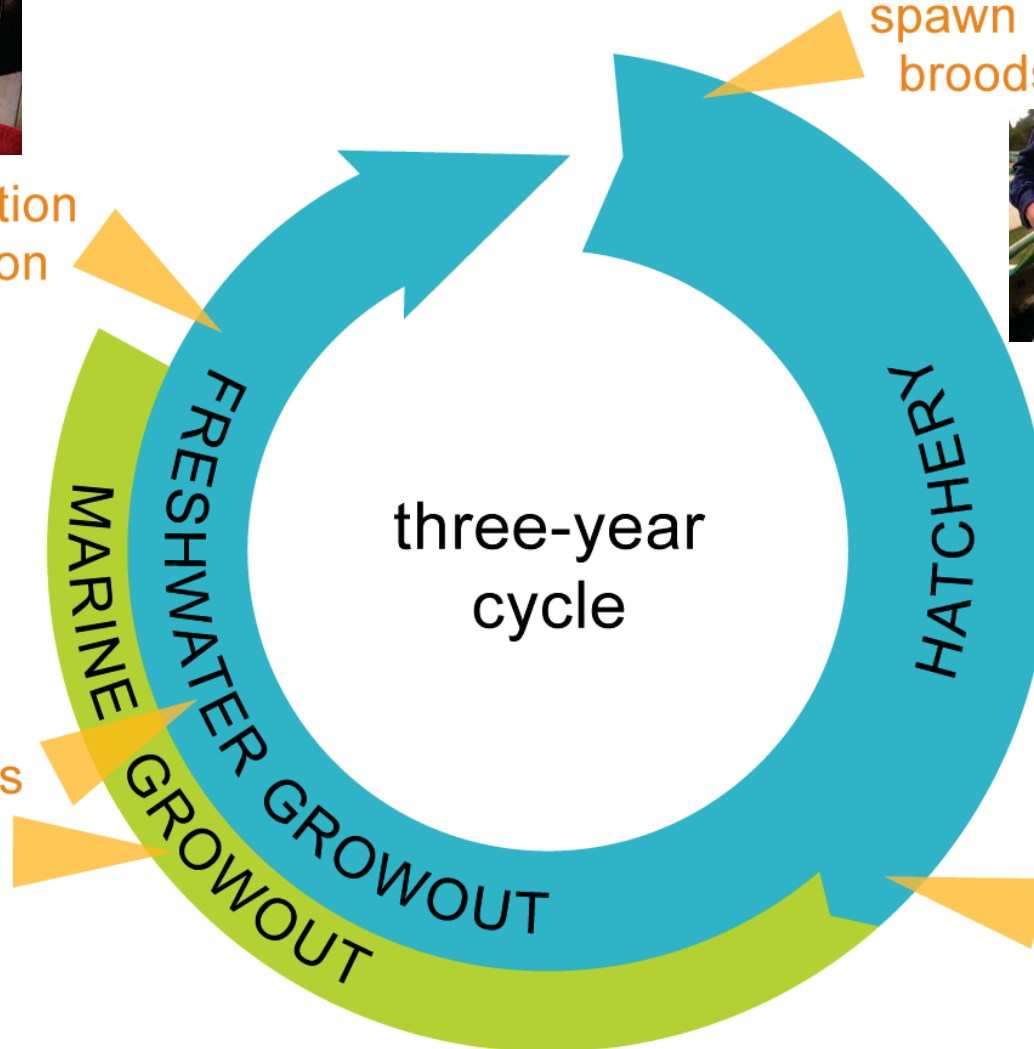
measurements



spawn selected  
broodstock



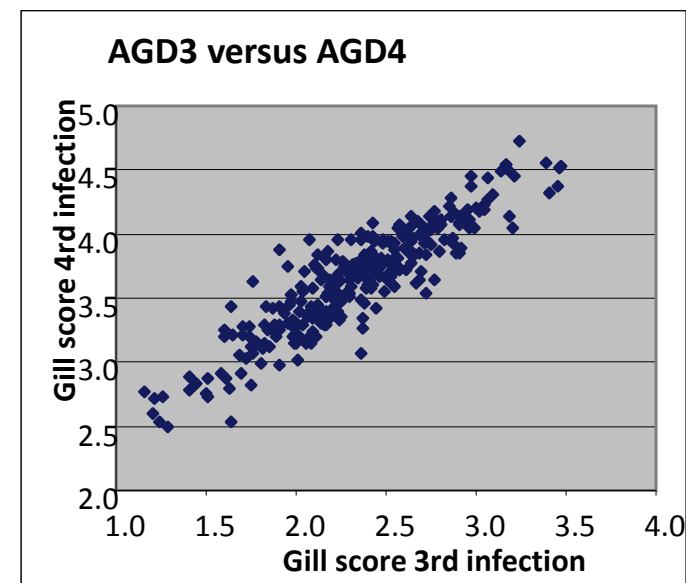
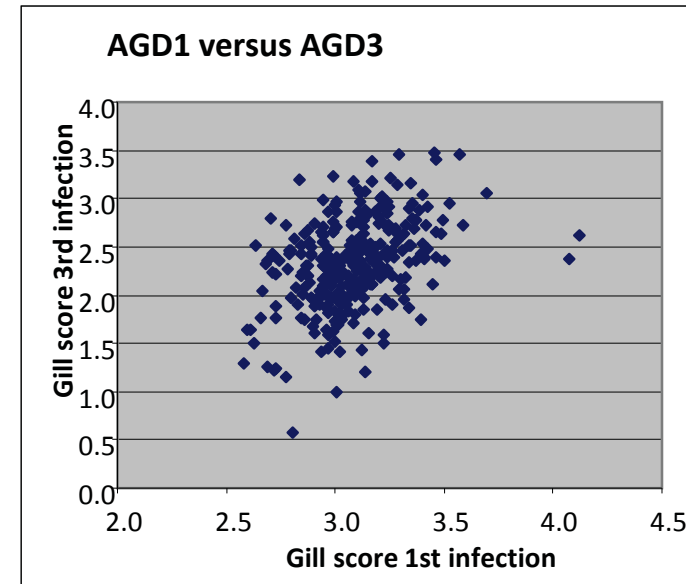
tag and DNA  
fingerprint



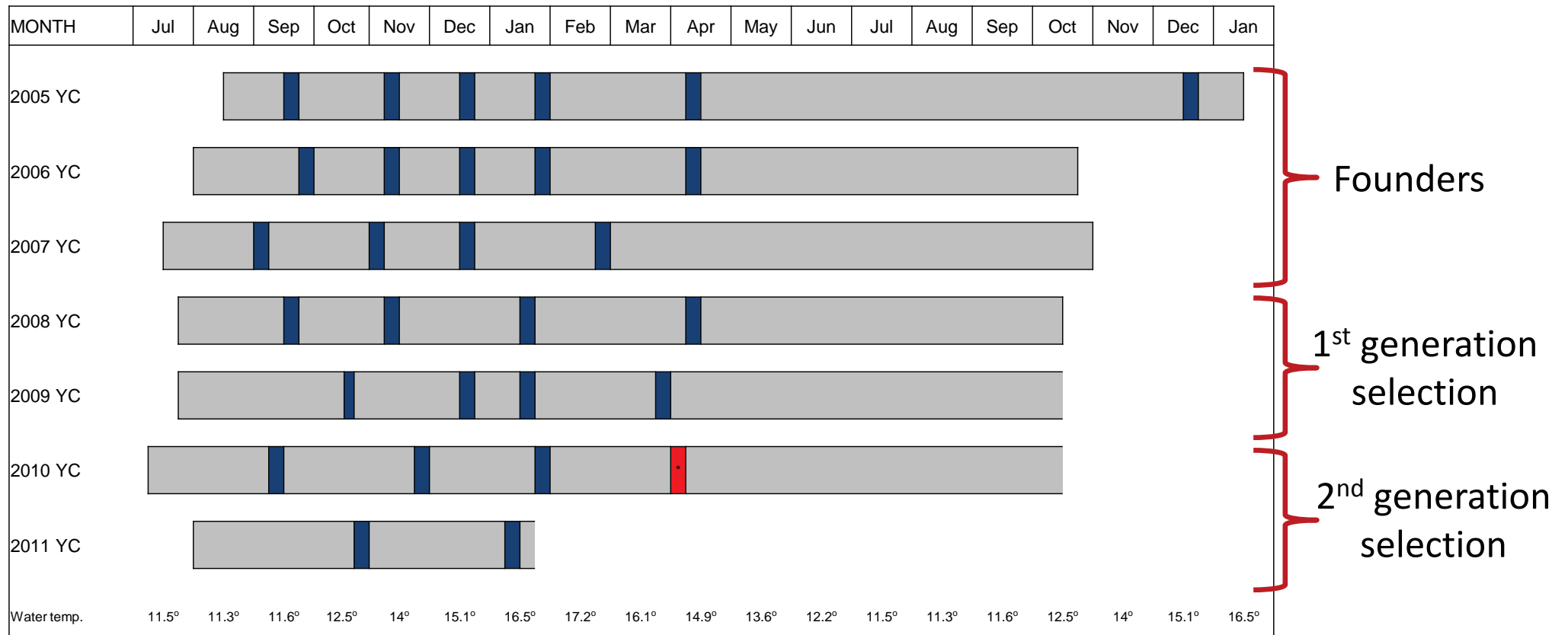


# Gill Score is a consistent heritable trait

- All infections have significant genetic variation
  - (heritability range is  $h^2 = 0.14 \pm 0.02$  to  $0.40 \pm 0.03$ )
- Two distinct traits, one is measured at first infection and the other at all subsequent infections
- First infection has lower genetic variation ( $h^2 = 0.14$ )
- Subsequent infections have stronger genetic expression, especially when measured during summer



# Selection is reducing the number of bathes

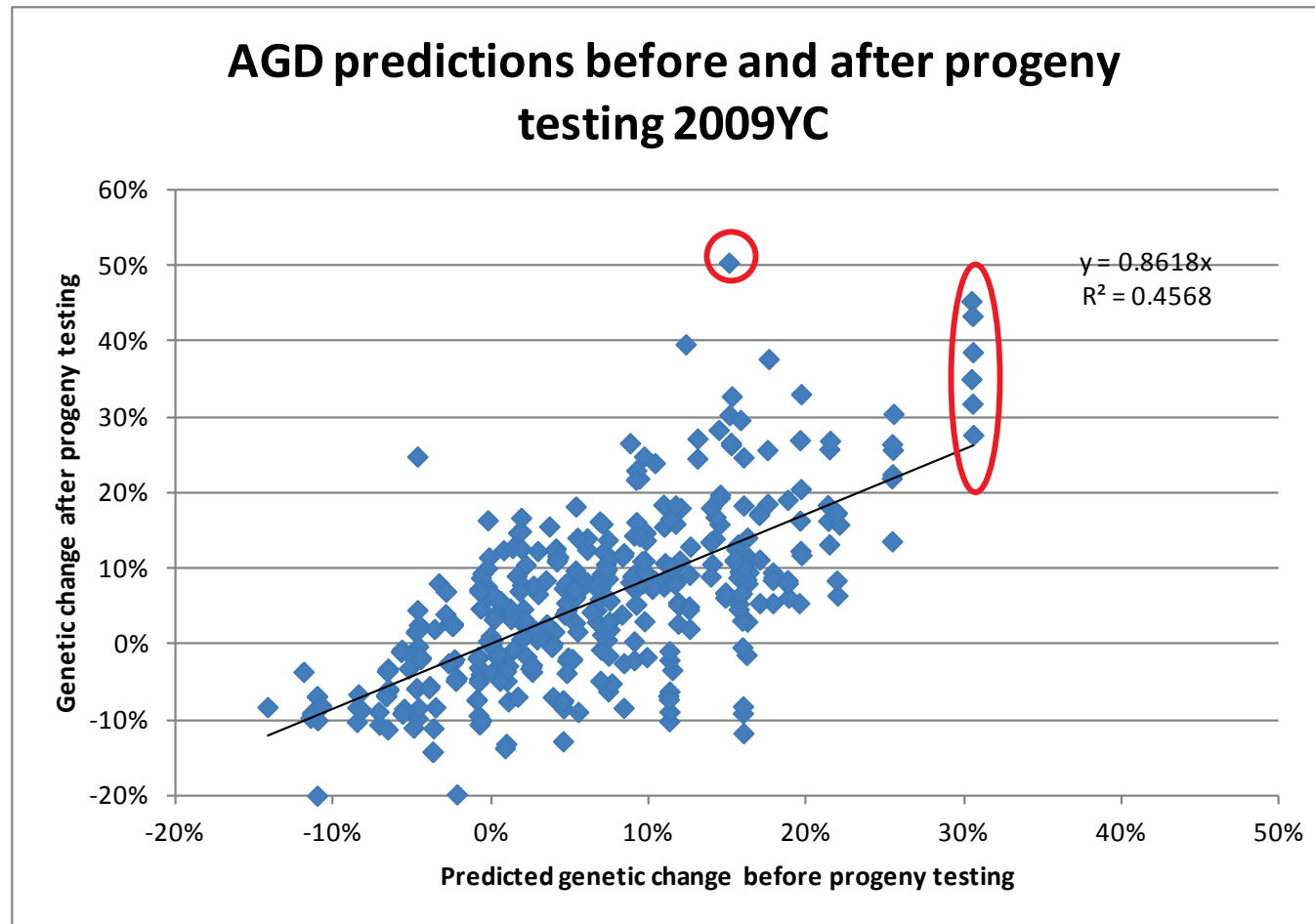


# Selection is working – A deliberate ‘Bad’ family

<b>AVERAGE FAMILY VALUES 2012 YC (SBP COHORT)</b>			
	<b>SBP families</b>	<b>BAD AGD family</b>	<b>Best AGD family</b>
EBV AGDac (Bath interval) *	25%	-12%	49%
Mean AGD1 Gill score	1.4	1.8	1.2
Mean AGD2 Gill score	3.1	4.5	1.2

**NB: Following GS2, Best AGD Family  $>75\% \leq$  GS1**

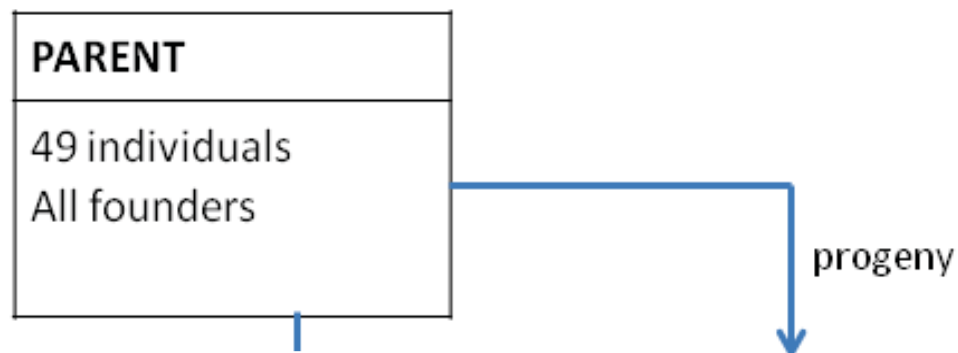
# Within vs between family variation



- Highlights the power of whole genome selection (WGS) to increase genetic gains as the broodstock remain in freshwater (biosecurity)



# Can SNP explain some of the AGD resistance observed?



SNP #	LG	%Variance
1	Ssa-07	34.85
2	Ssa-01	22.45
3	Ssa-19	15.16

REFERENCE	Used for:
1,348 individuals 2005-2007 YC 49 half-sib families • Min: n=10 • Max: n=80 • Mean: n=39 Data: ➤ AGD phenotypes ➤ Genotypes	QTL Analysis
	Producing EBV for BROODSTOCK
	Producing DGV/GEBV for BROODSTOCK

# Summary

- Activities are focused on 'HOST', 'PATHOGEN' and 'ENVIRONMENT'
- Focus is on delivering solutions/information to assist in alleviating the issue
- Partnership with the end users (industry) is crucial
- AGD R&D needs to be dynamic to adjust to changing priorities
- International collaboration is key – no need to reinvent the wheel

# The CSIRO AGD Team

## ***Amoeba Biology***

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Richard Taylor

Natasha Botwright

Paula Lima

## ***AGD Resilience***

**Richard Taylor**

Peter Kube

## ***Selective Breeding***

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Peter Kube

Richard Taylor

Mat Cook

Sonja Dominik

John Henshall

Scott Cooper

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Sharon Appleyard

Paula Lima

## ***Dietary Intervention***

**Brett Glencross**

Mat Cook

Ben Maynard

Richard Taylor

# Funding bodies and Industry and Academic collaborators



AUSTRALIAN  
SEAFOOD  
COOPERATIVE  
RESEARCH CENTRE



FISHERIES  
RESEARCH &  
DEVELOPMENT  
CORPORATION



Flinders  
UNIVERSITY  
ADELAIDE • AUSTRALIA



# Thank you

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