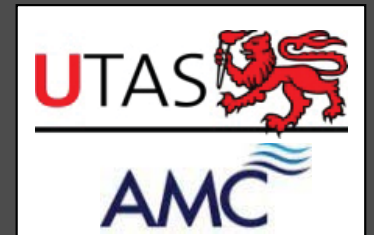


AGD research at the University of Tasmania

Barbara Nowak

NCMCRS
AQUATIC ANIMAL HEALTH RESEARCH GROUP

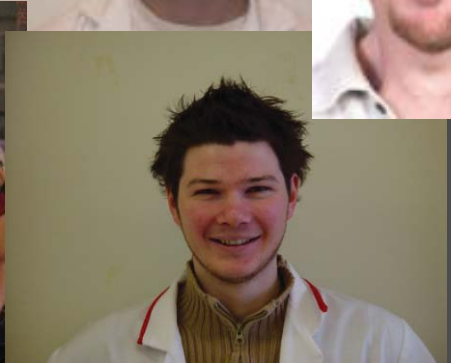
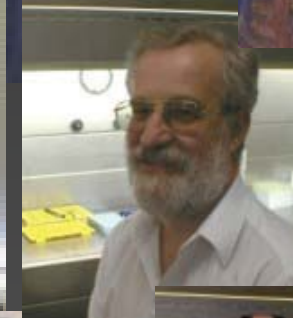
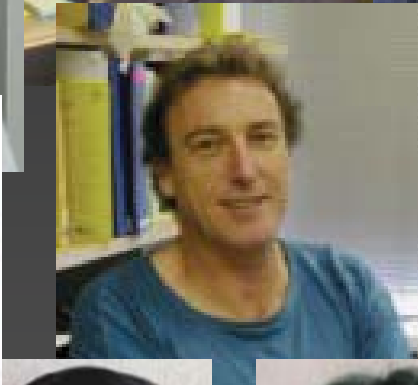


AGD



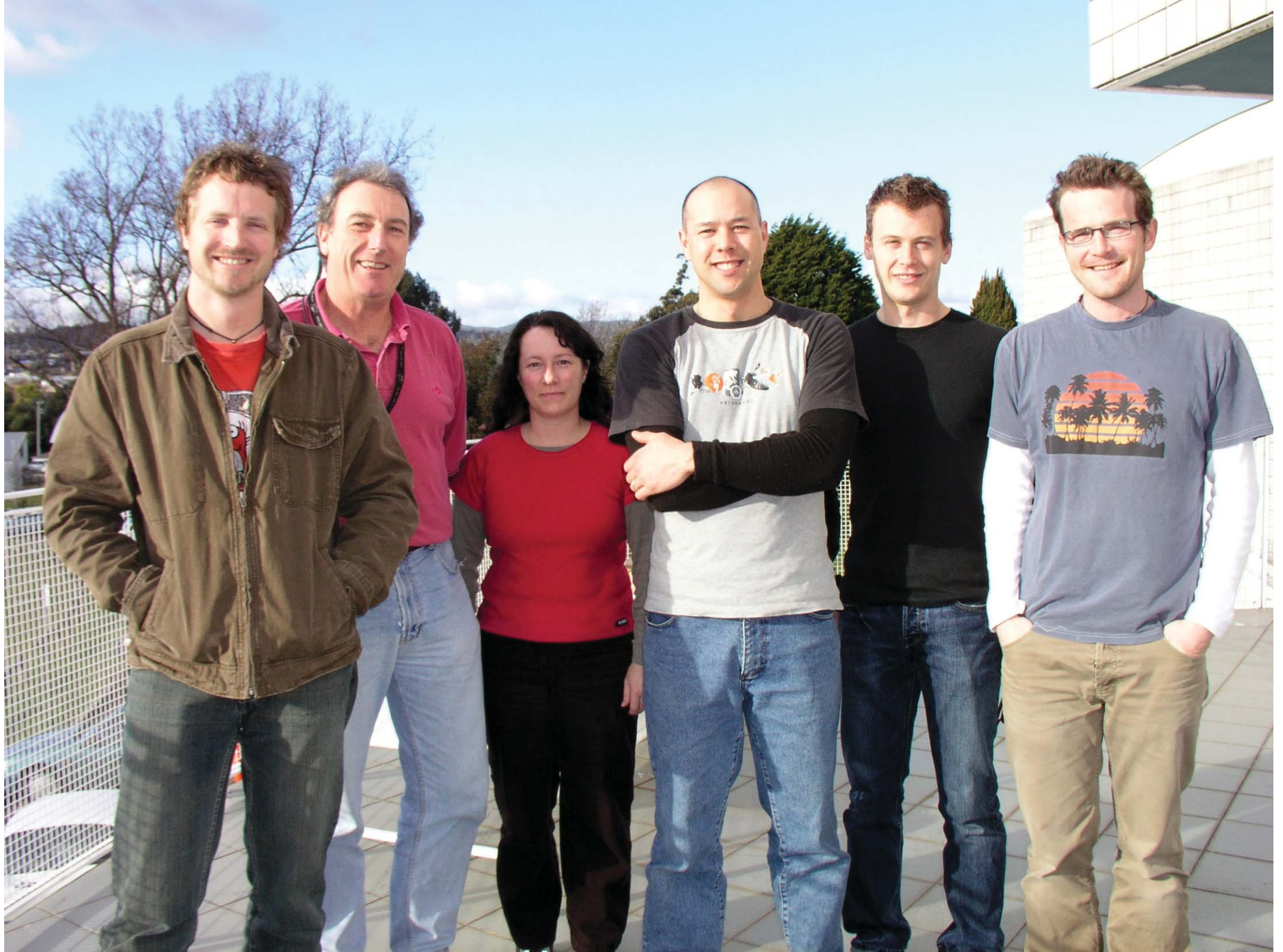


AGD team

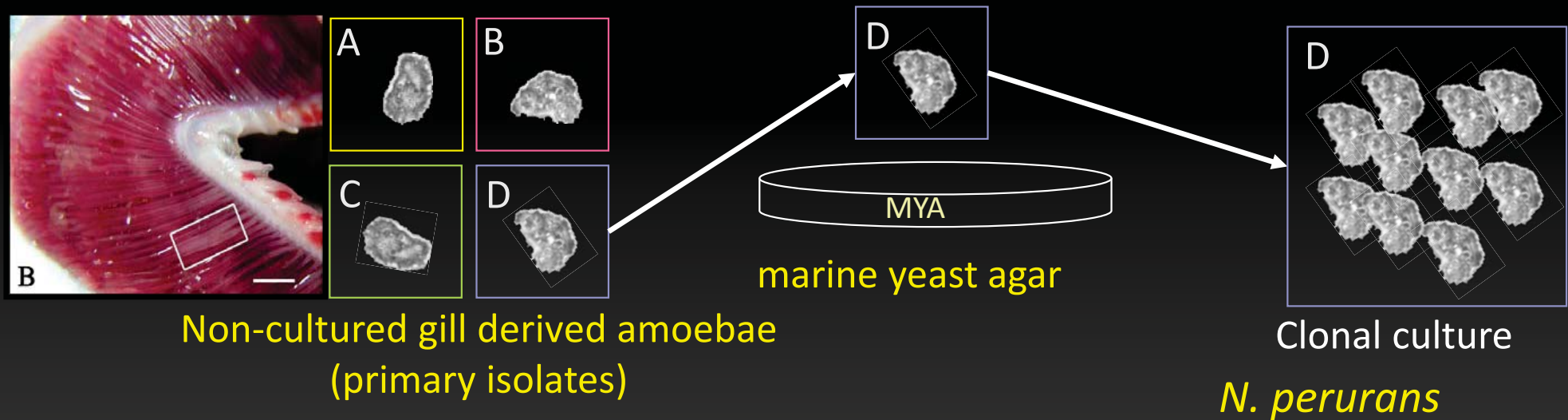


International collaborations





Koch's postulates fulfilled for *N. perurans* as an aetiological agent of AGD



Clonal culture infectious after 70 days
(Crosbie et al 2012)

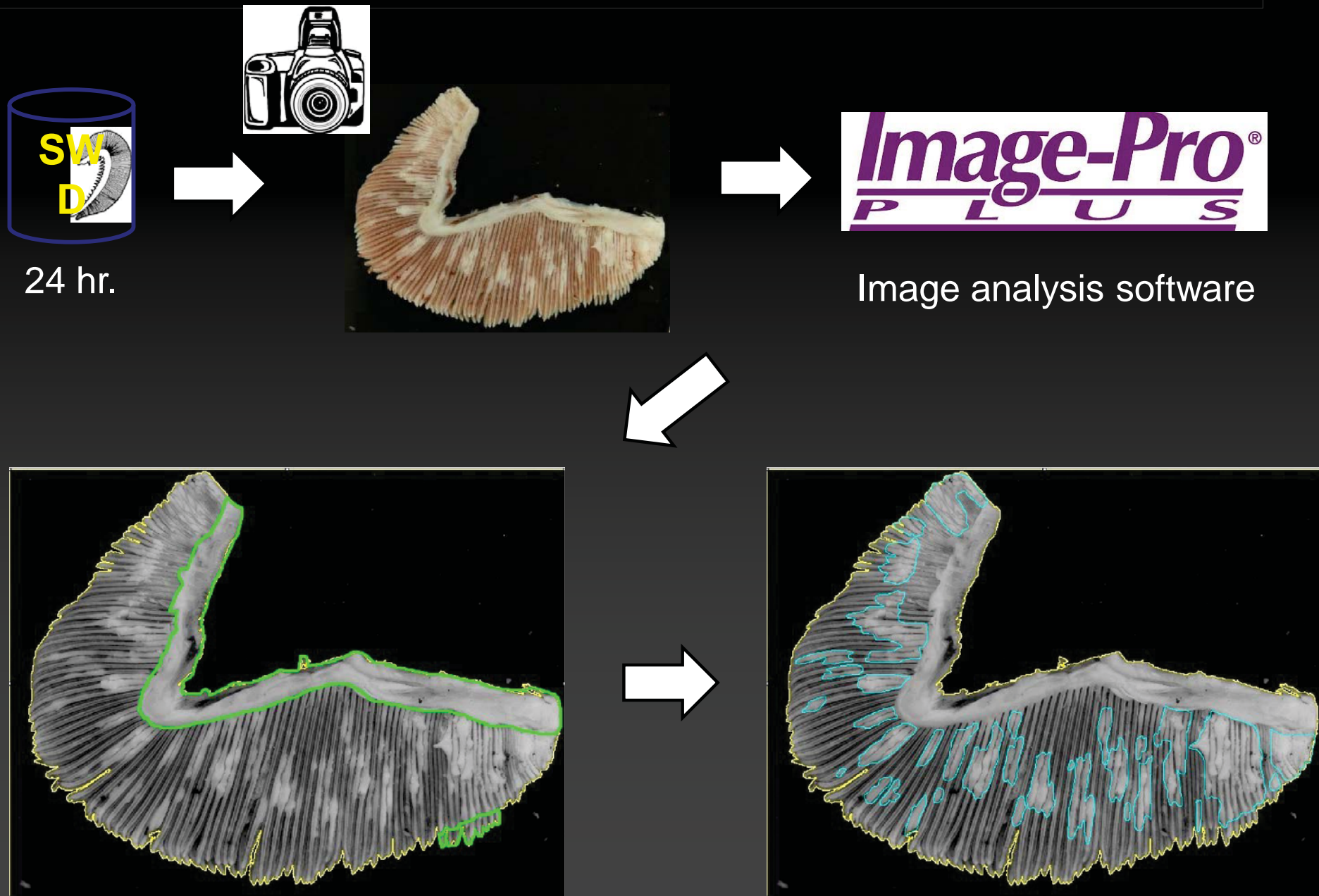


**Aquatic Animal Health
Research Group
NCMCRS, UTAS**

Current AGD research

- Determination of AGD severity
- AGD in vitro model
- Determination of *N. perurans* in water
- Management through behaviour manipulation
- Challenge dynamics
- Treatments
- Susceptibility of other species
- Immune response

Determination of AGD severity



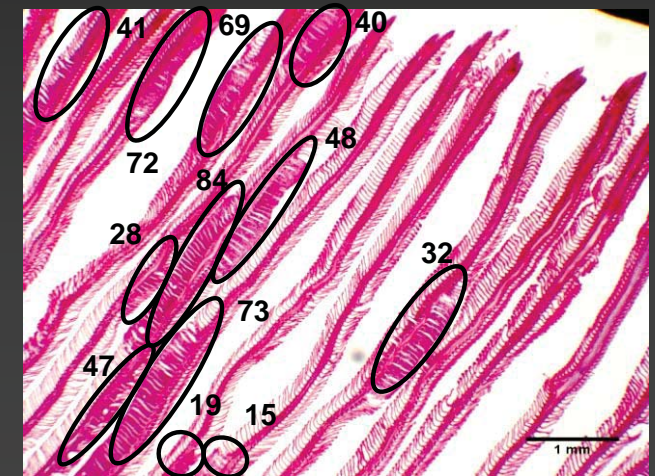
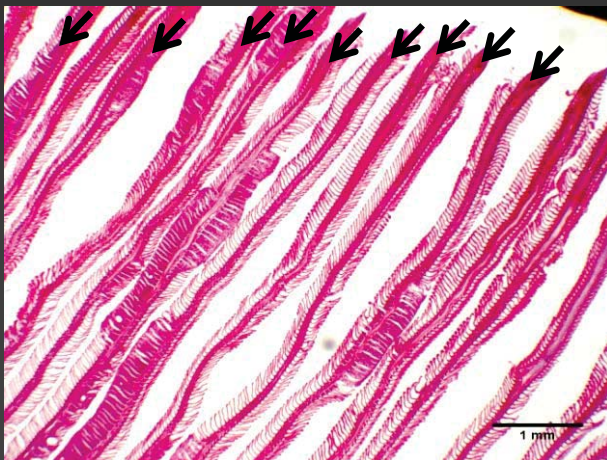
Histological severity assessments



% Affected filaments

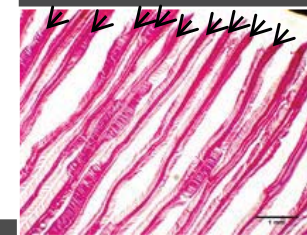
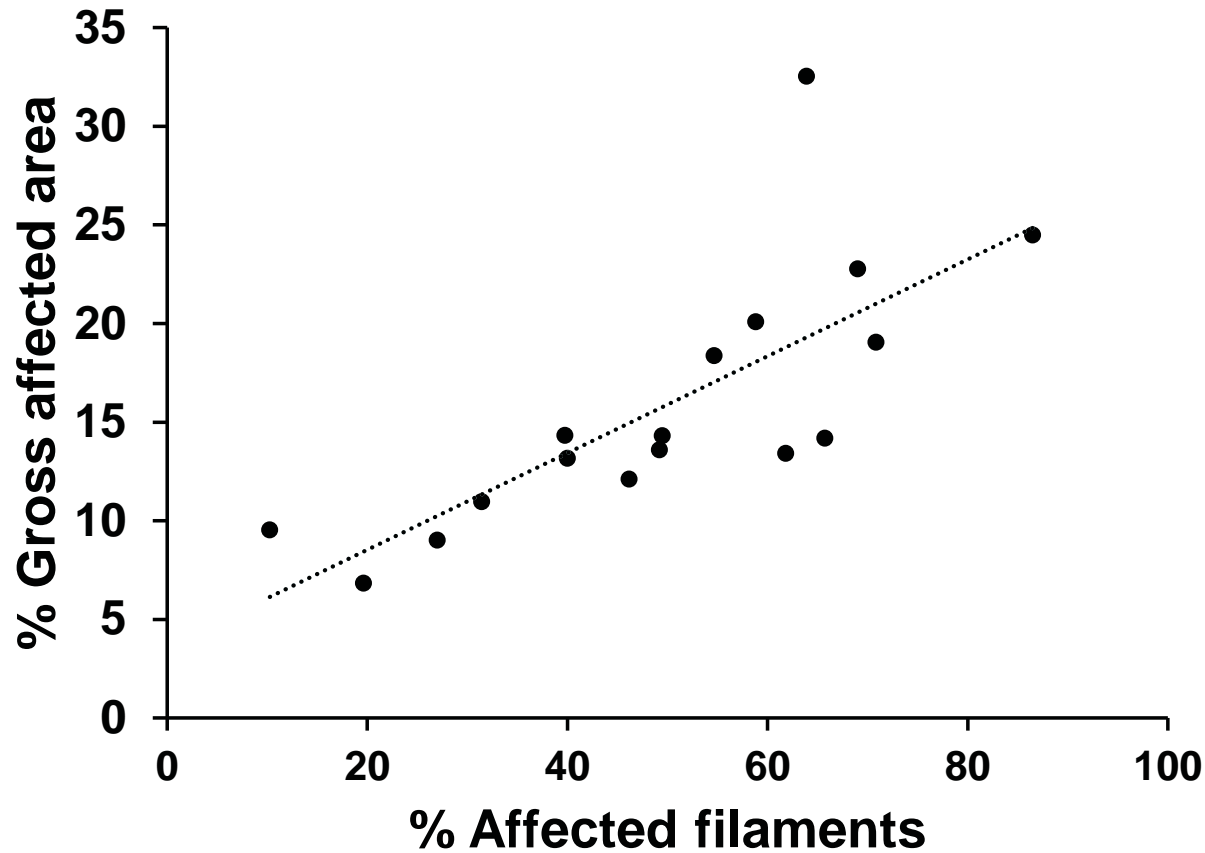
No. of lesions/affected filament

Lesion size

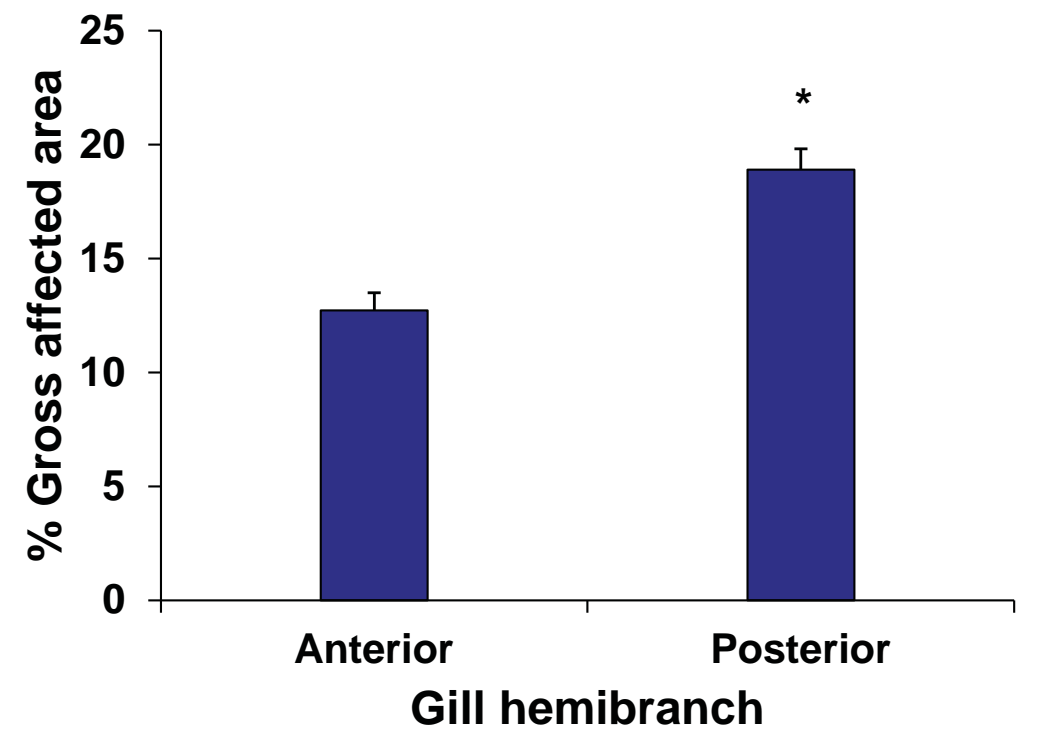
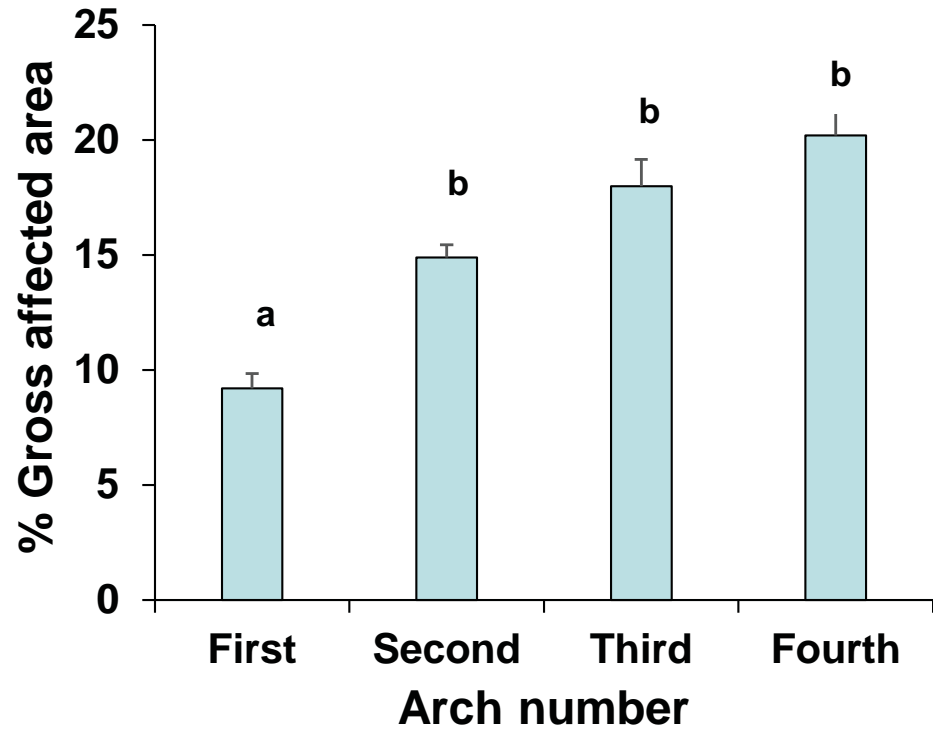


Determination of AGD severity

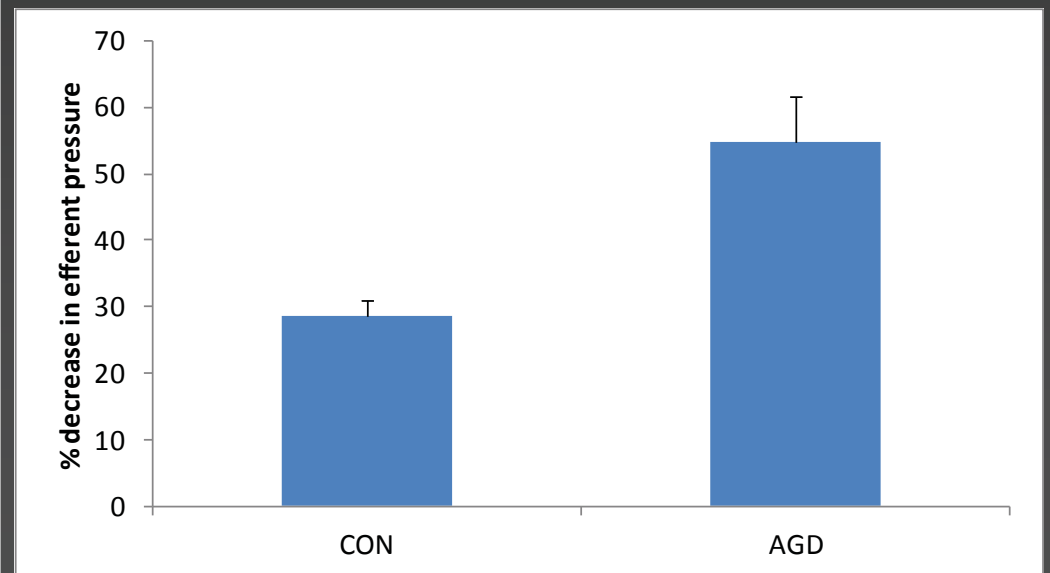
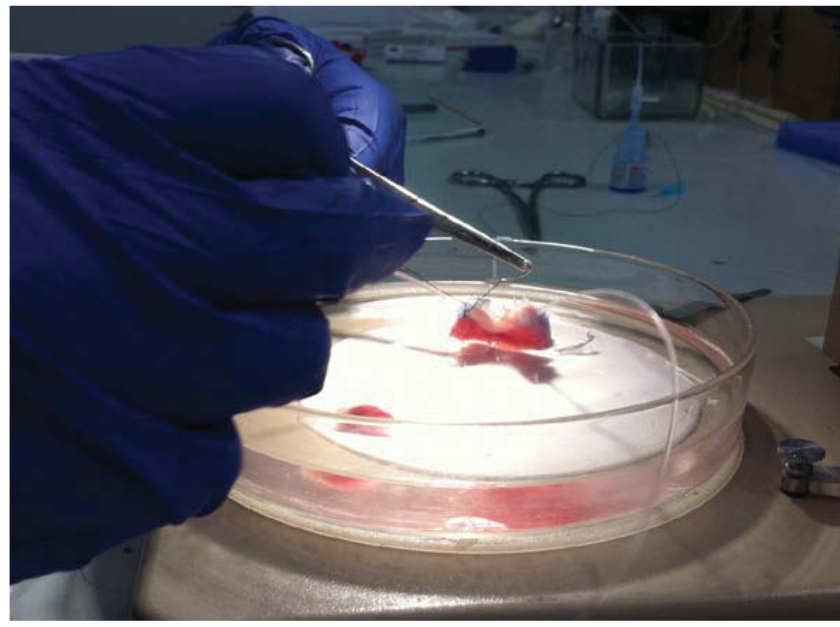
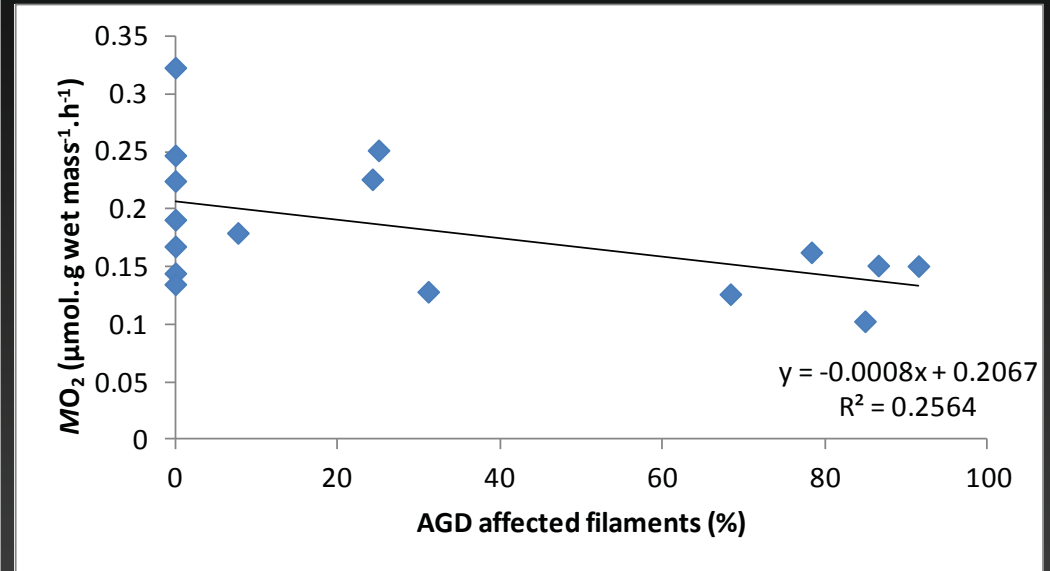
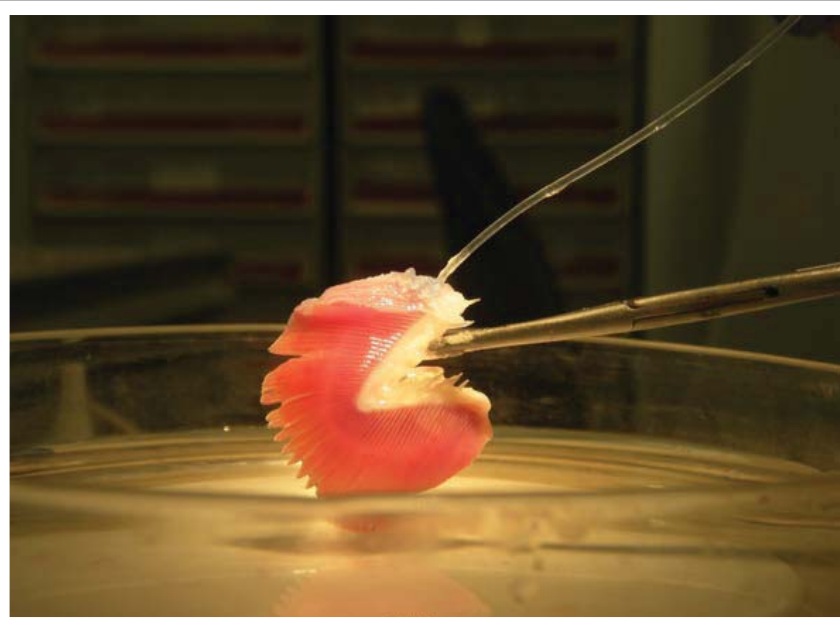
$y = 0.31x + 0.16$
 $r^2 = 0.62$
 $r = 0.79, n=17$
 $F=24.39, df=1, 15,$
 $P<0.001$



Determination of AGD severity



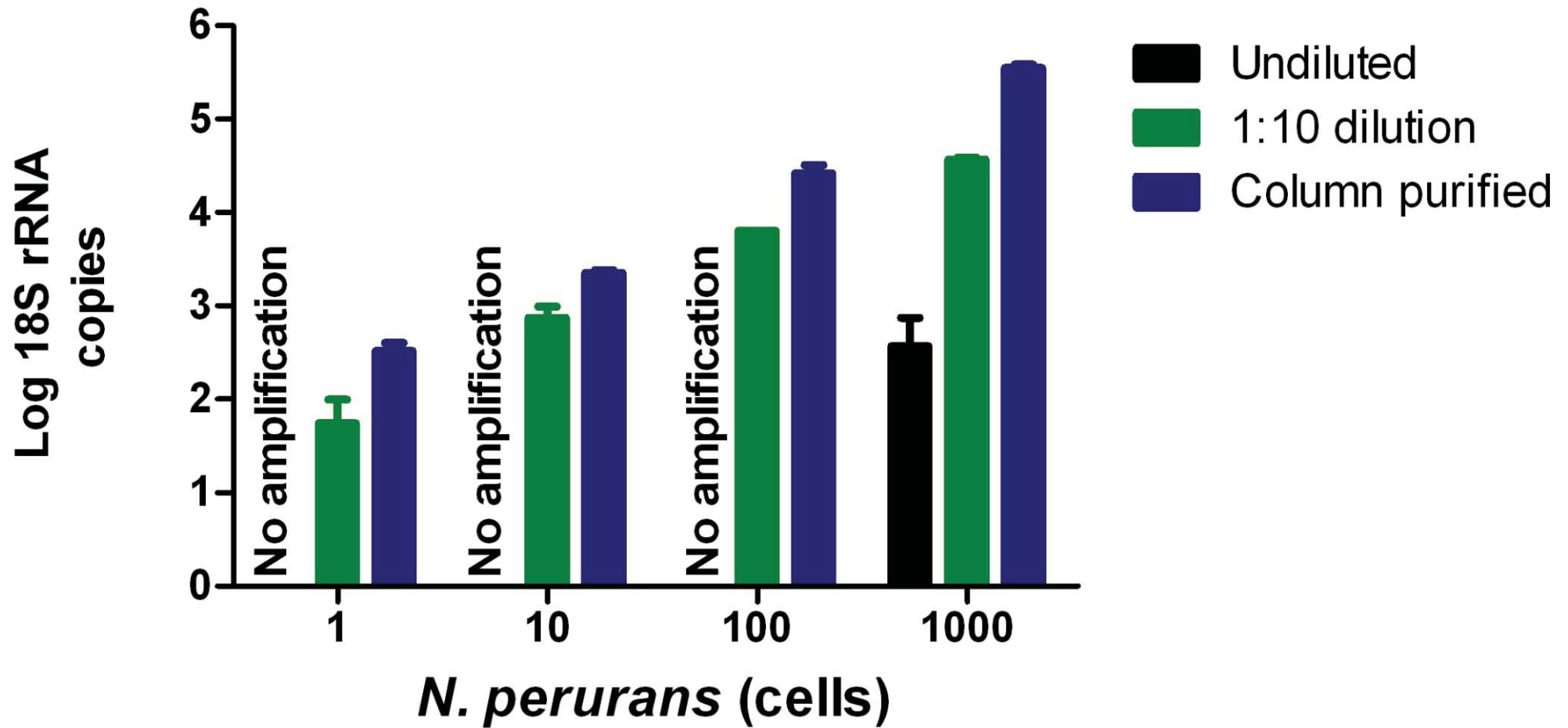
In vitro gill model for AGD

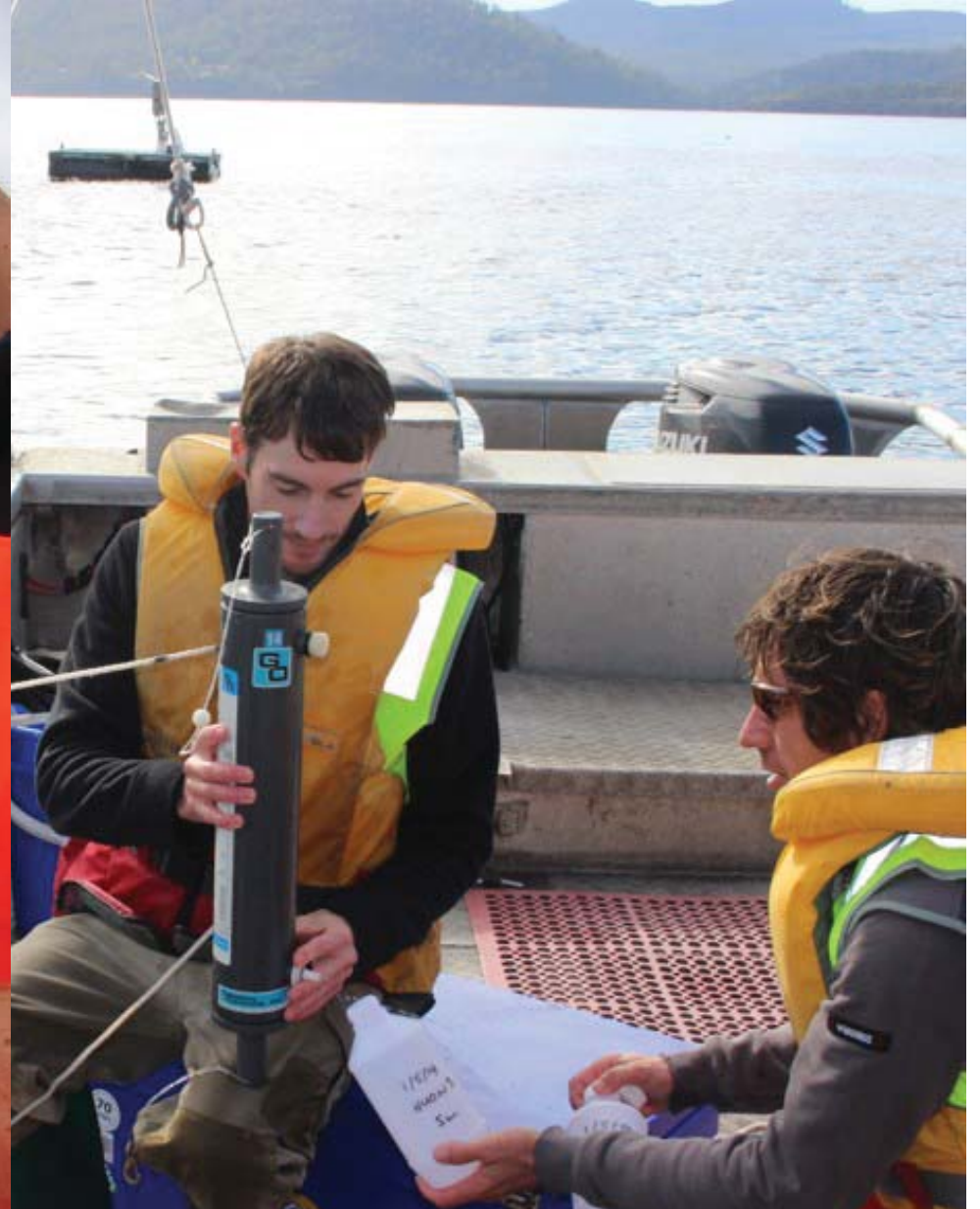


Detection of *N. perurans* in water samples



Inhibitory effects of algae

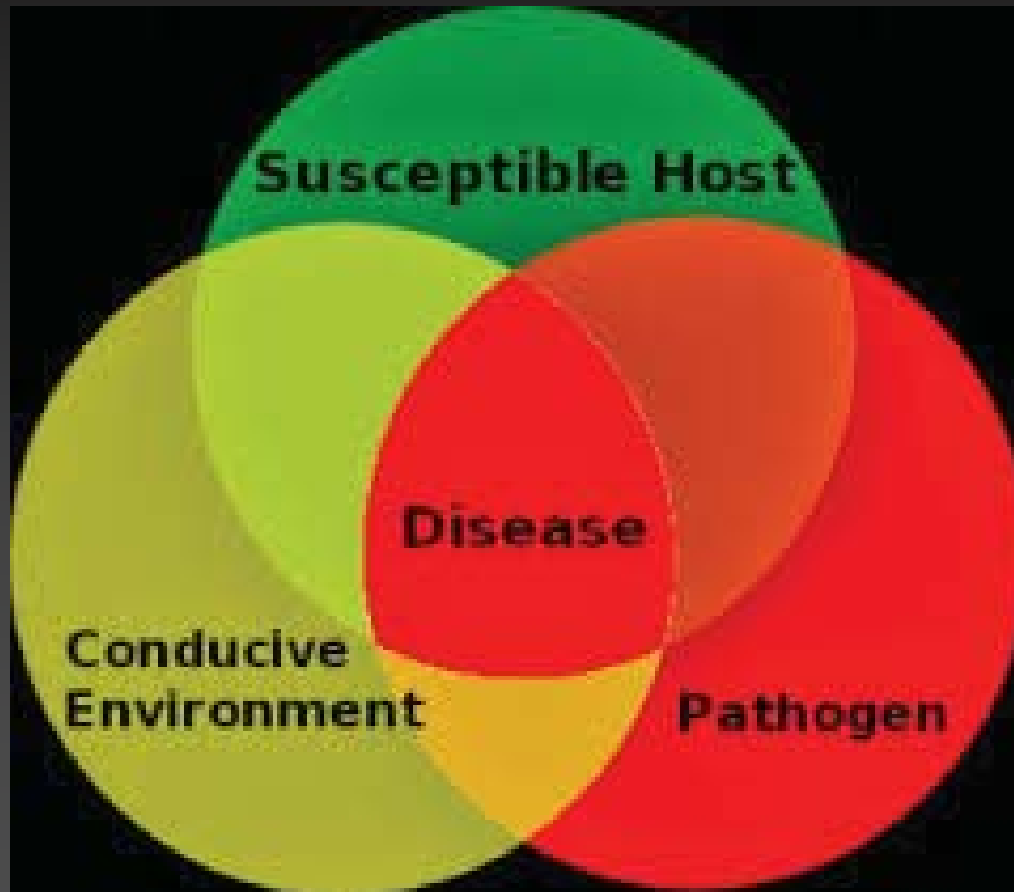




THE UNIVERSITY OF

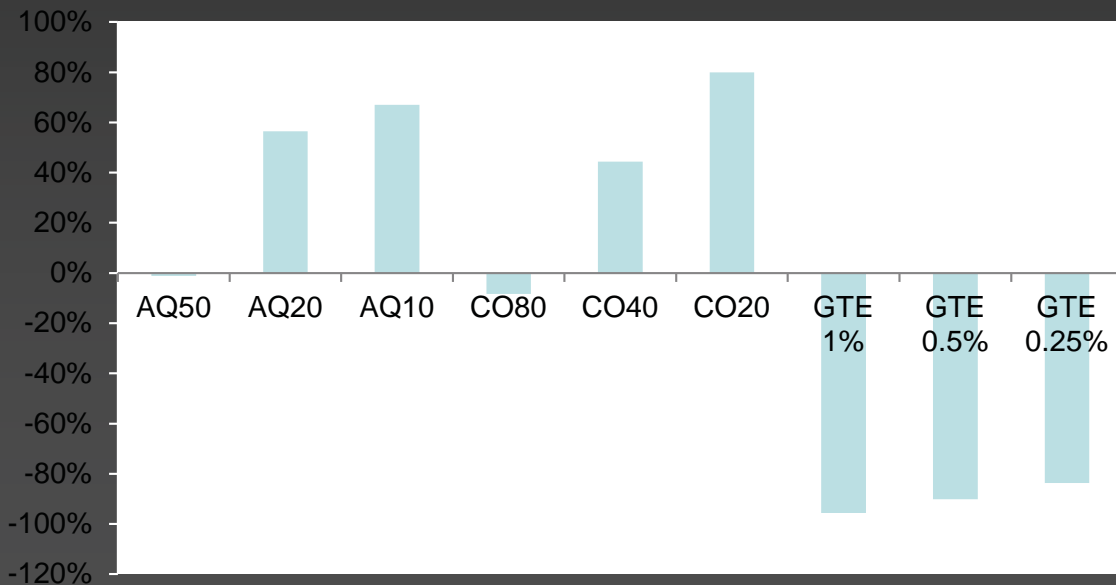
MELBOURNE

AGD challenge dynamics

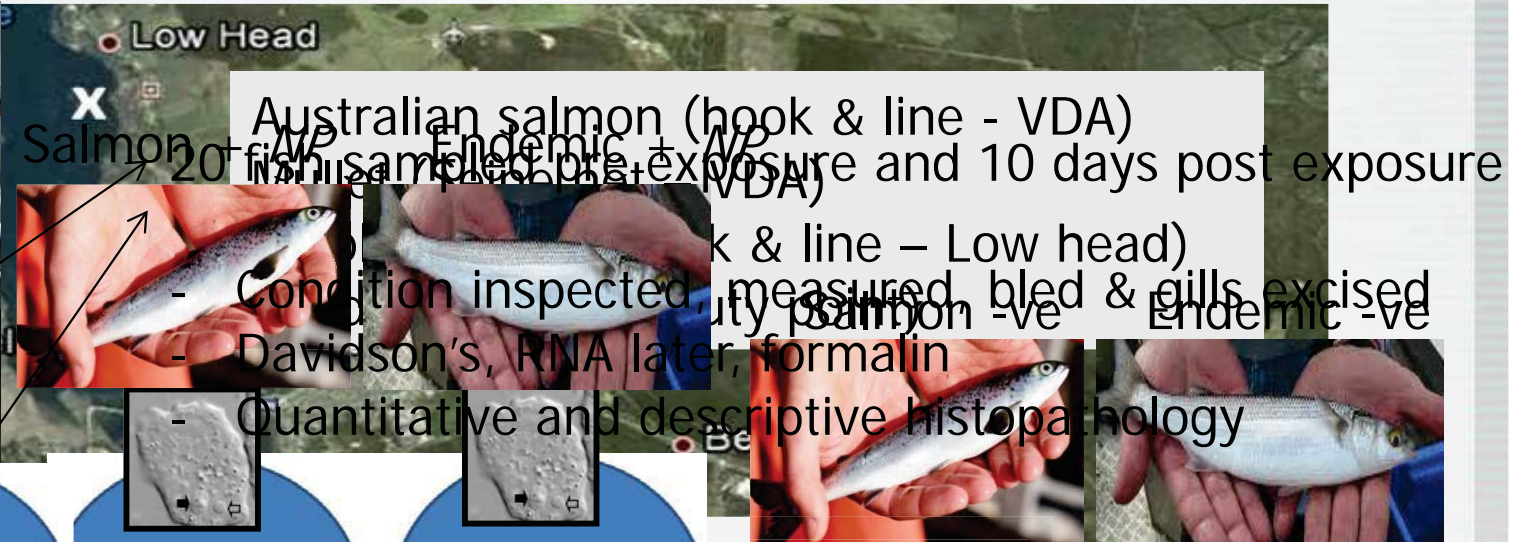


Treatments

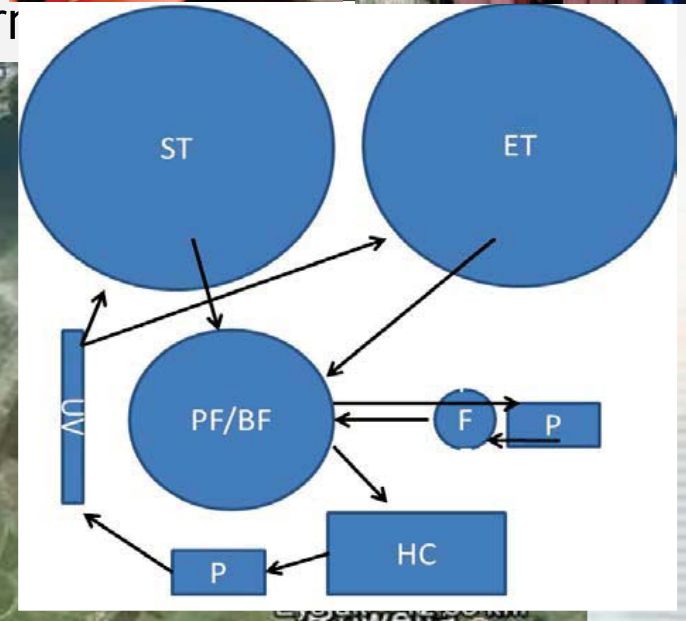
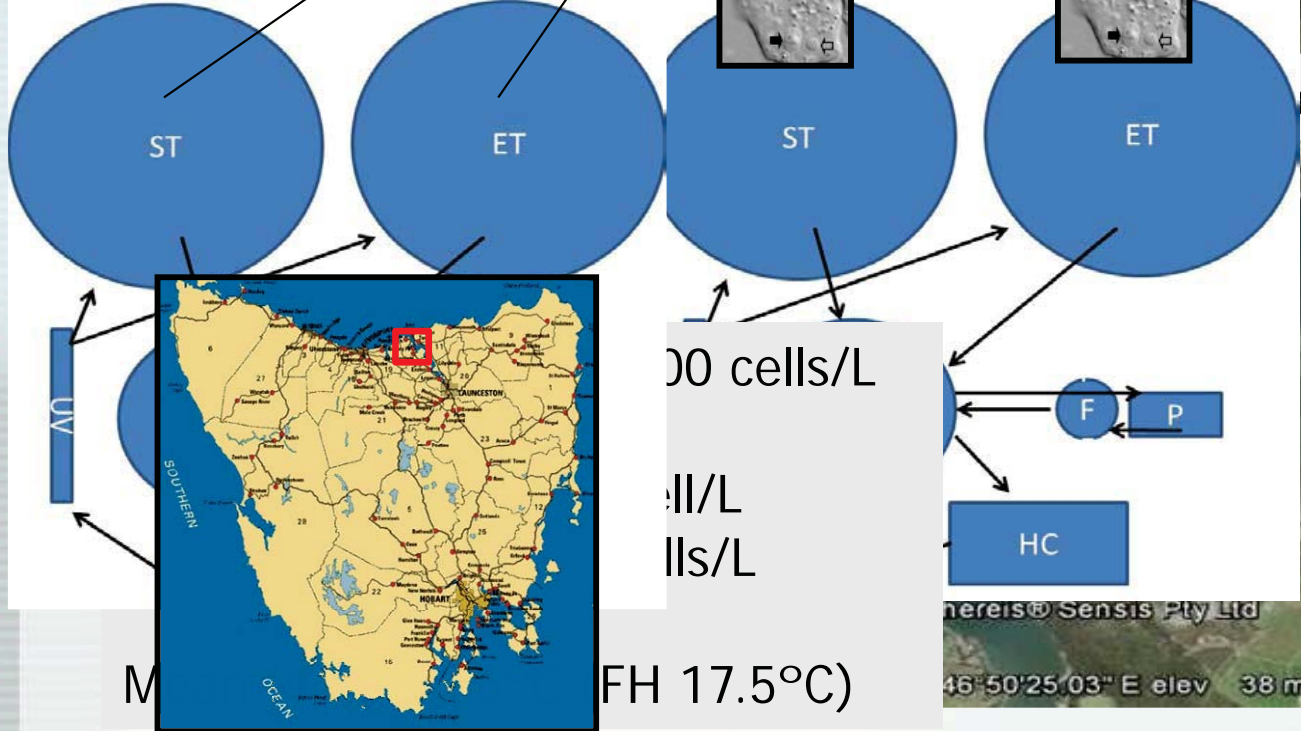
- hydrogen peroxide
- novel treatments



Experimental Design (Screening Trials)



Australian salmon (hook & line - VDA)
 Salmon 20 fish sampled pre exposure and 10 days post exposure
 Endemic + (VDA) / Endemic -ve (VDA)
 Hook & line - Low head
 - Condition inspected, measured, bled & gills excised
 - Davidson's, RNA later, formalin
 - Quantitative and descriptive histopathology



Endemic fish susceptibility to AGD

- Results (Purple Wrasse)

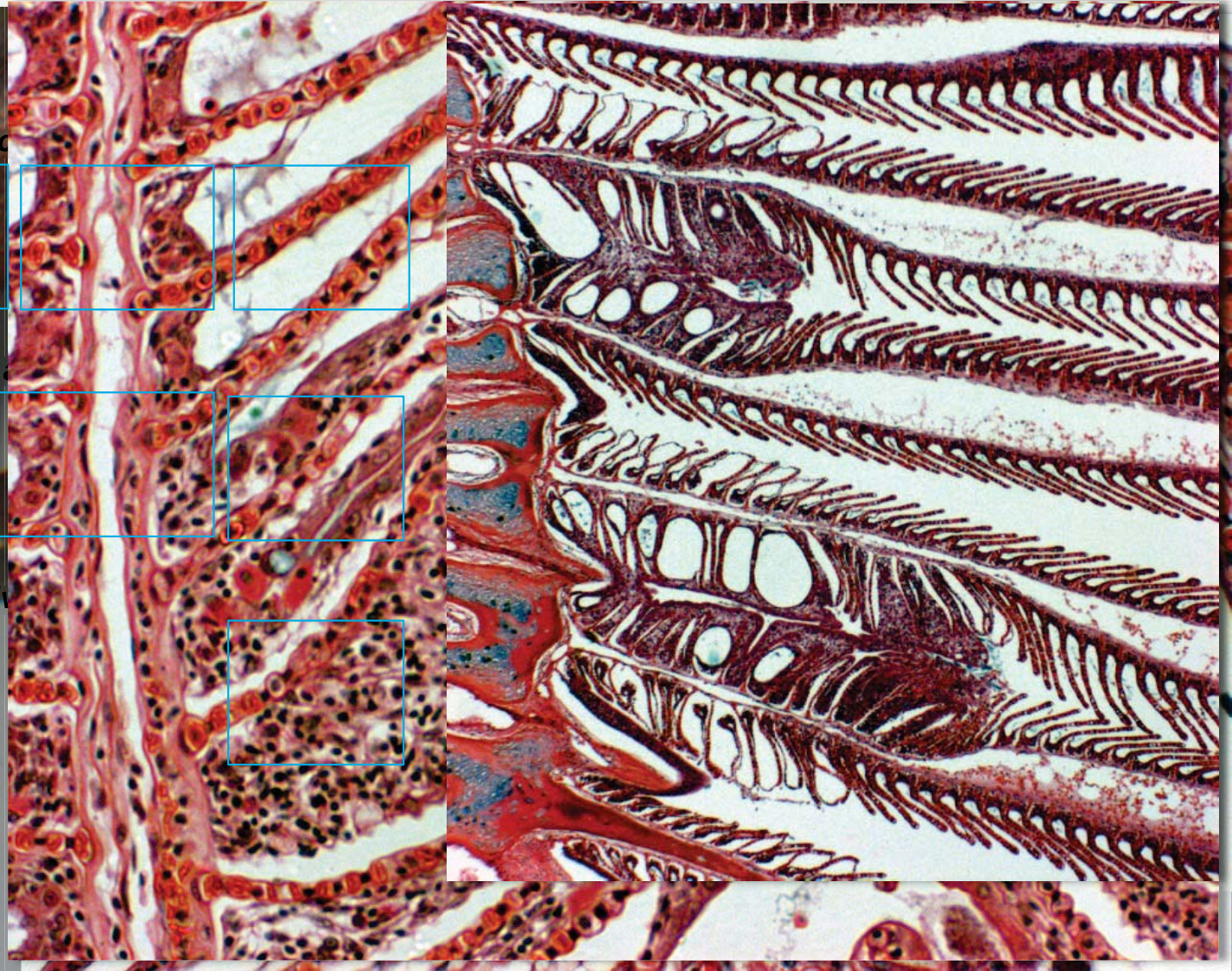
Notolus pucicola



% Filaments with hyperplastic lesions	
Species	Pre-exposure
Atlantic salmon	0
Wrasse	0

% Filaments with <i>N. perurans</i>	
Species	Pre-exposure
Atlantic salmon	0
Wrasse	0

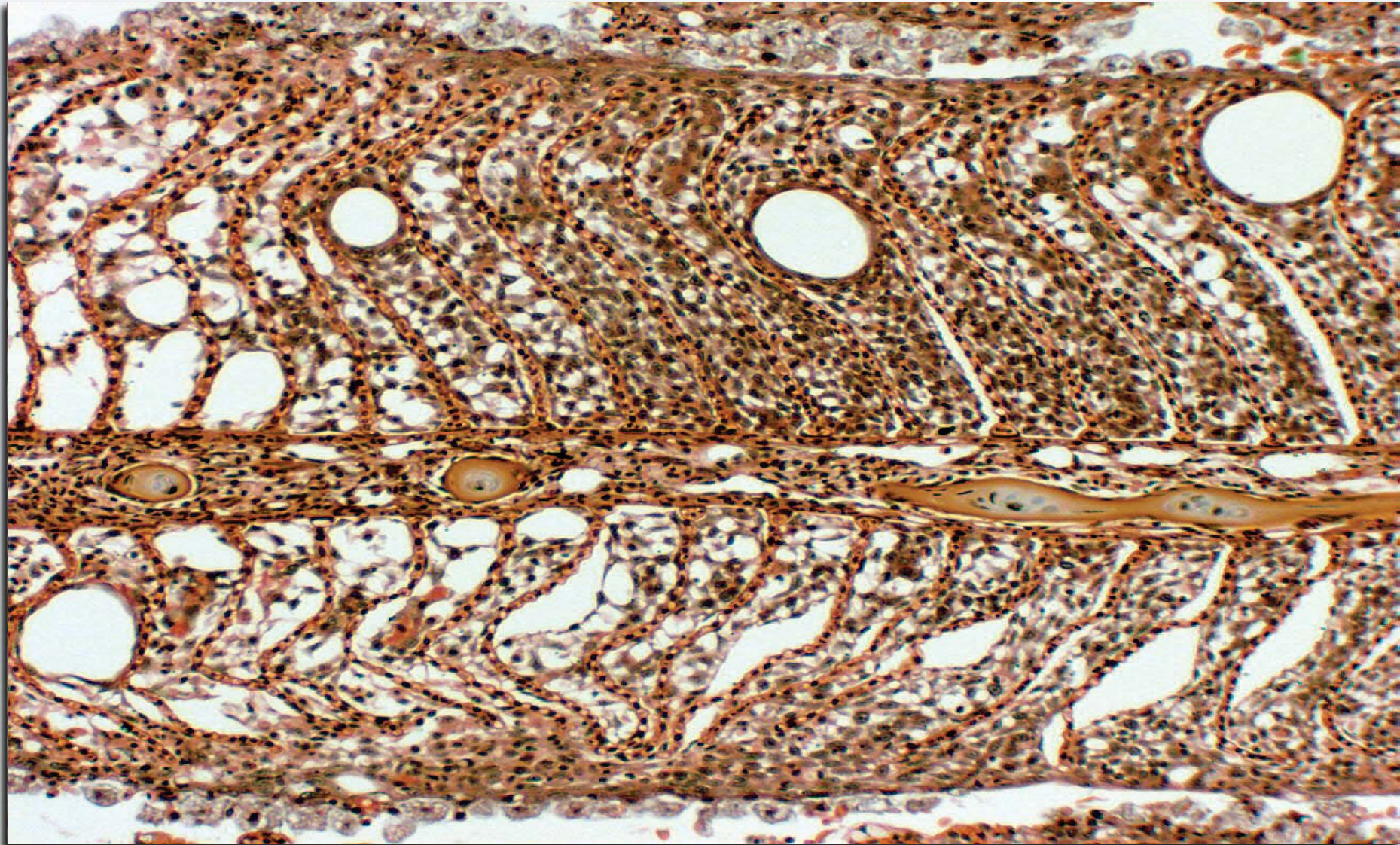
% Hyperplastic lesions positive for <i>N. perurans</i>	
Species	Pre-exposure
Atlantic salmon	0
Wrasse	0



Endemic fish susceptibility to AGD

- Results (Southern sand flathead)

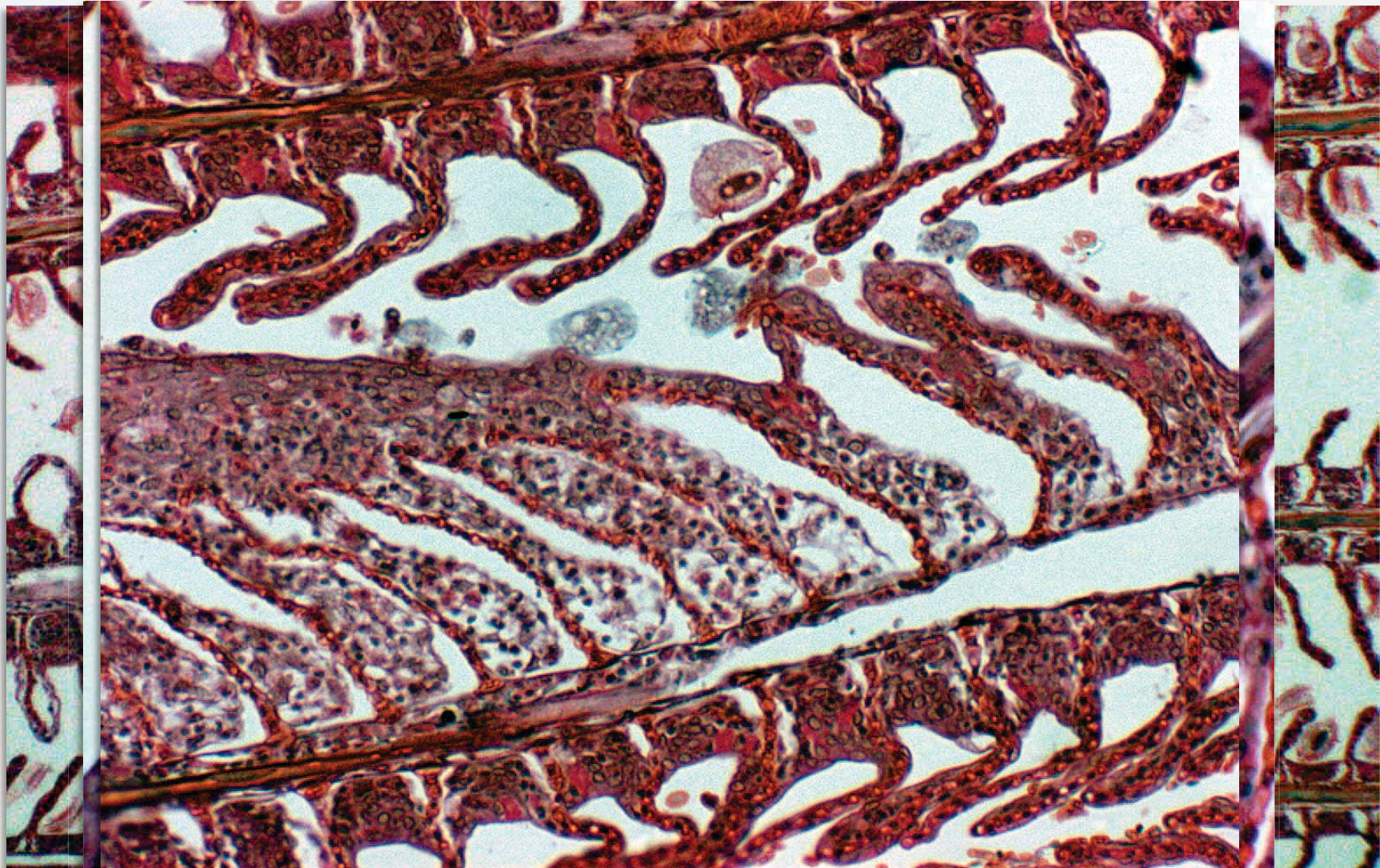
Platycephalus bassensis



Endemic fish susceptibility to AGD

- Results (Australian salmon)

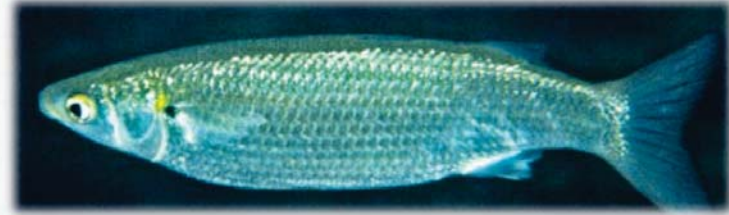
Arripis trutta



Endemic fish susceptibility to AGD

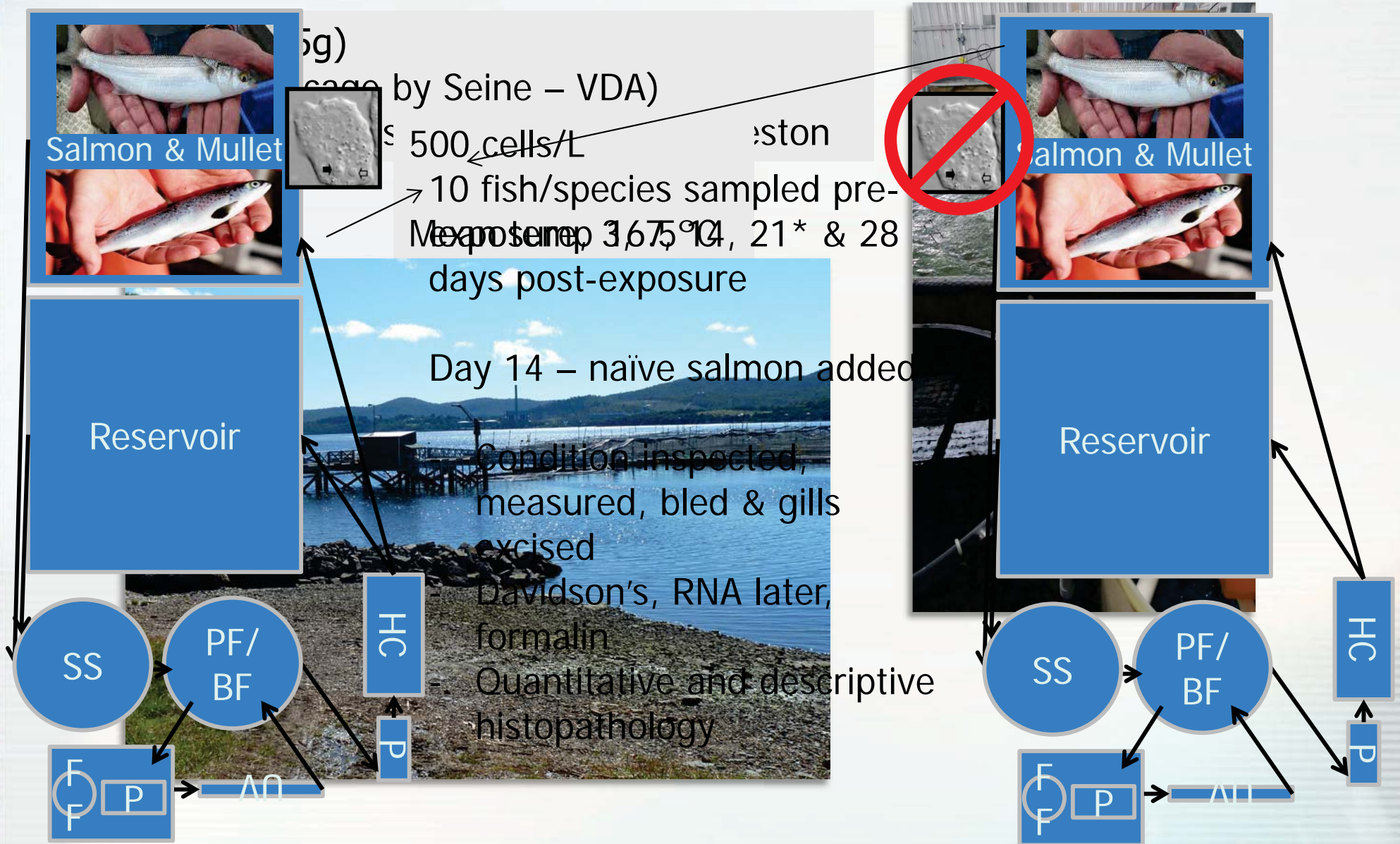
- Results (Yellow eye mullet)

Aldrichetta forsteri



Endemic fish susceptibility to AGD

- Experimental Design (Extended mullet challenge)

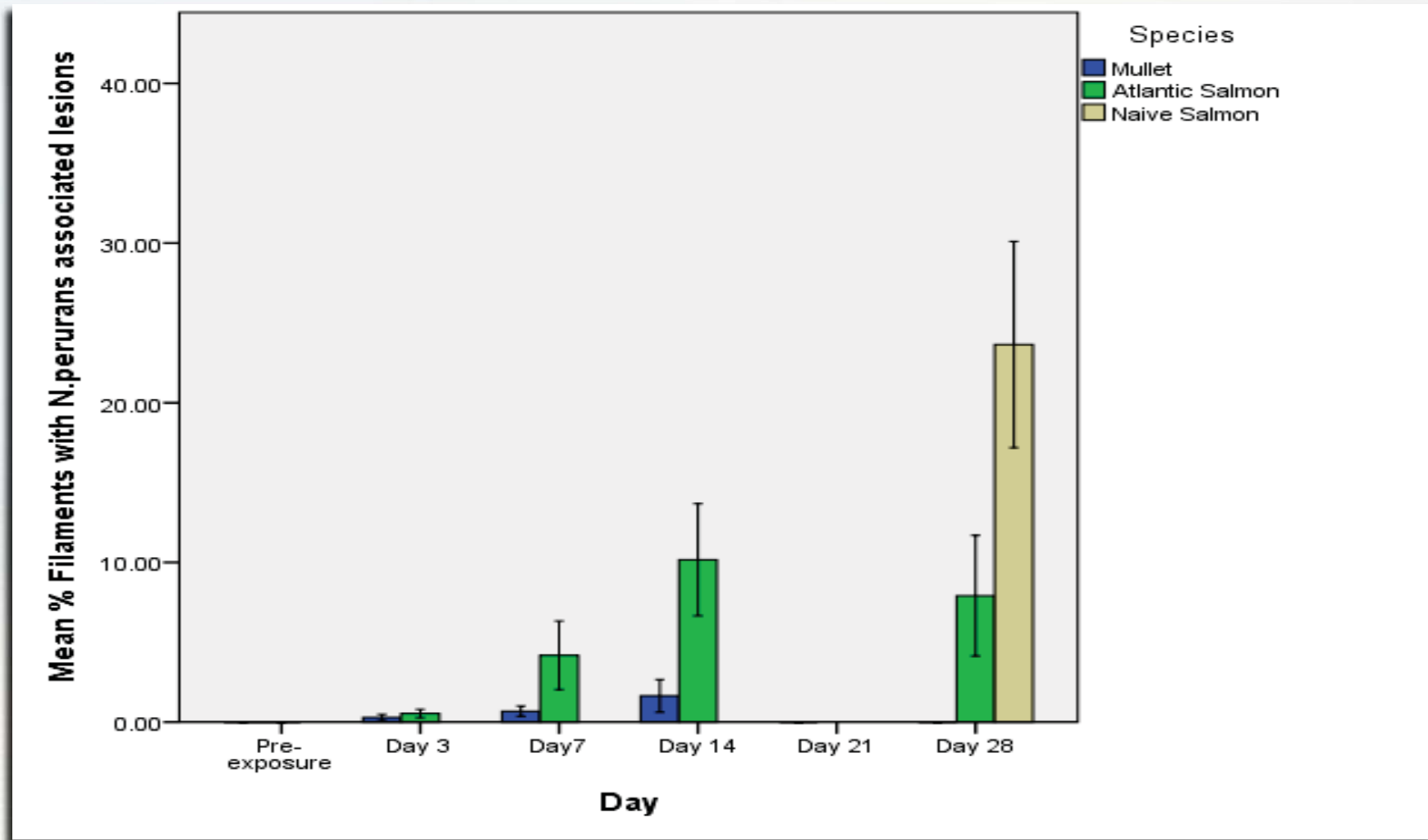


Endemic fish susceptibility to AGD

- Results (Extended mullet challenge)

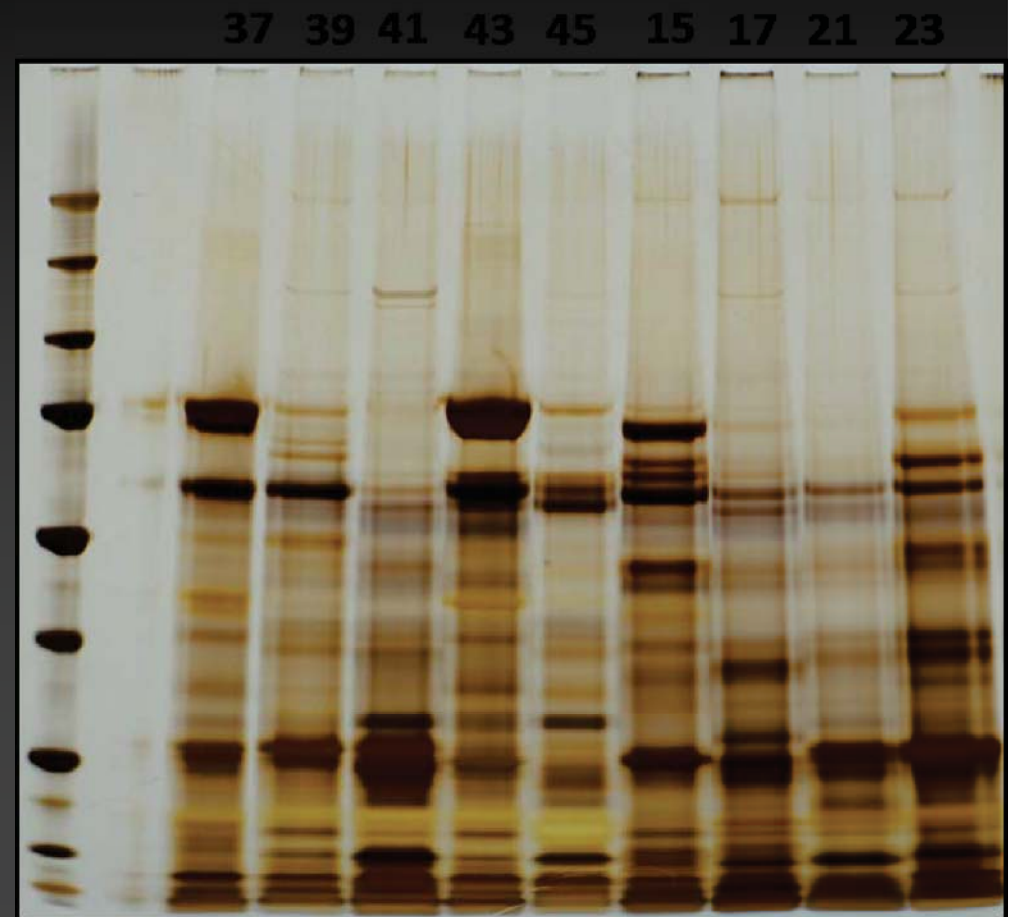


Infected fish



Proteomics of mucus

- nanoLC MS/MS used to characterize changes in mucus proteome of AGD-affected Atlantic salmon
- Salmon (n=10) exposed to *N. perurans* 4 times
- Gill and skin mucus collected

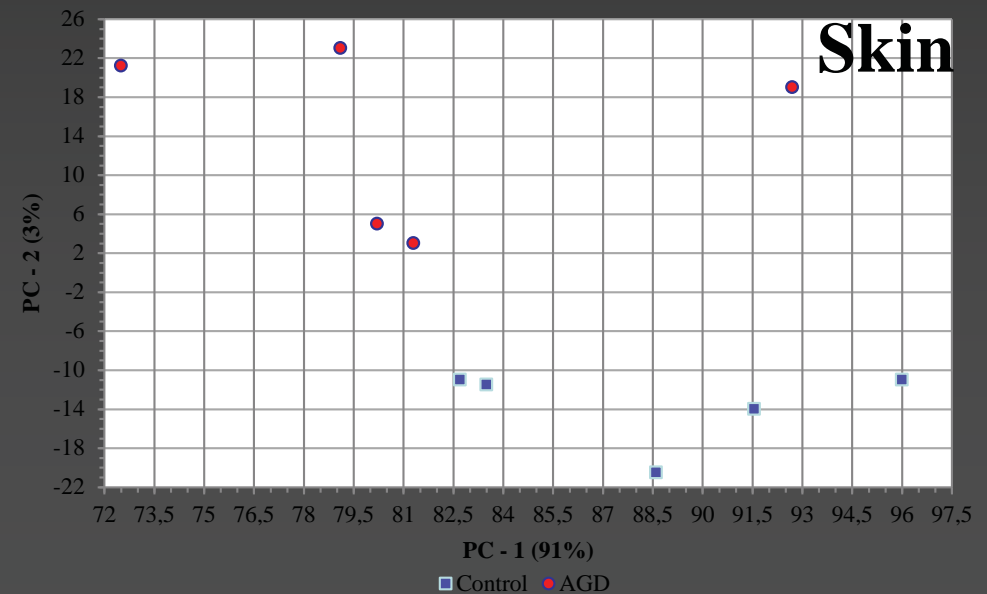
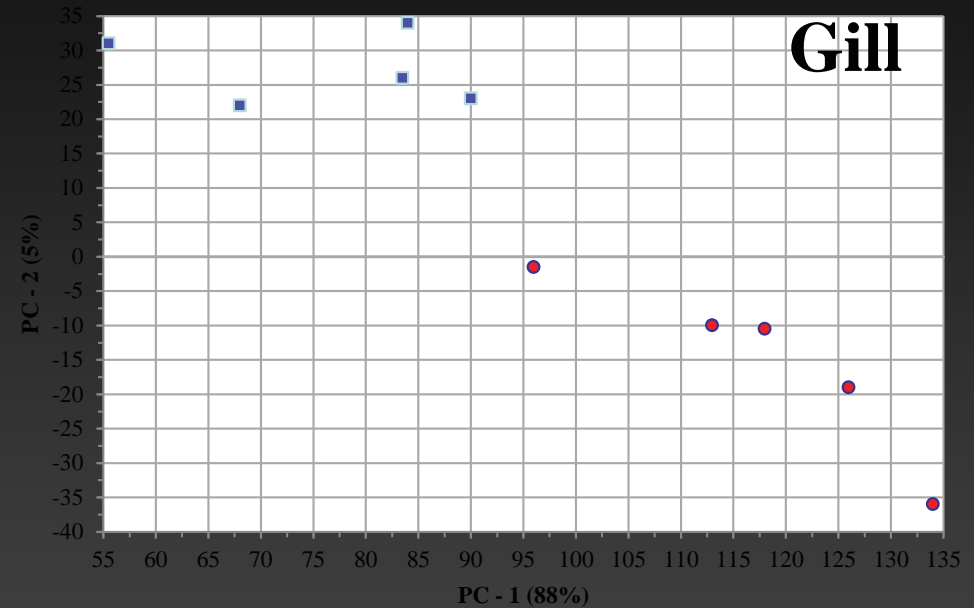


Proteins id by LC/MS

- Non- redundant protein groups
 - 186 in gill mucus
 - 322 in skin mucus
 - 15% of gill and 21% of skin mucus proteins were identified only in AGD samples
- Blood contaminants (serotransferrin and seroalbumin) removed from statistical analyses after peptide counts were normalized

Principal Component Analysis

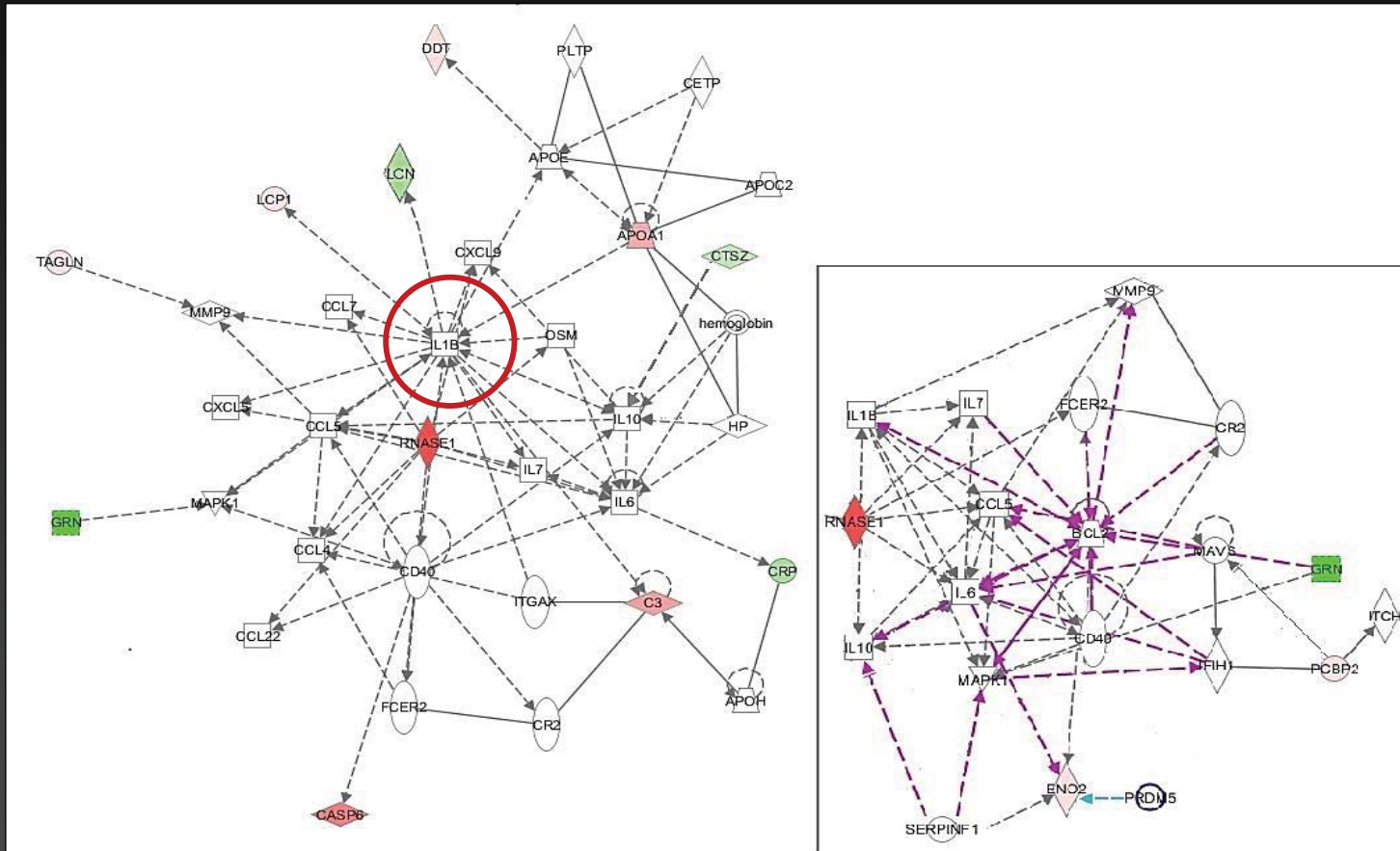
- Control and AGD-affected samples clustered together
 - PC2 (3%) effect for skin
 - PC1 (91%) and PC2 (5%) for gill



Proteins Identified

- Included
 - Immune and inflammation related proteins: C-reactive protein, Apolipoprotein-1, granulins, cathepsin
 - Factors previously associated with AGD: asAG-2, complement factor C3, S-100, FK506, annexin
- Ingenuity Pathway Analysis → map interaction networks of differentially expressed proteins
 - Gill samples in 3 noteworthy networks: “Cell to Cell Signalling and Haematological System Function”, “Infectious Disease” and “Cell Death and Survival”.
 - Proteins in networks → high level of interaction with IL-1 β (AGD-associated) and IL-6

Gill protein interaction networks



Coloured figures show statistically significant ($P < 0.05$) differential expression by beta-binomial distribution analysis in R

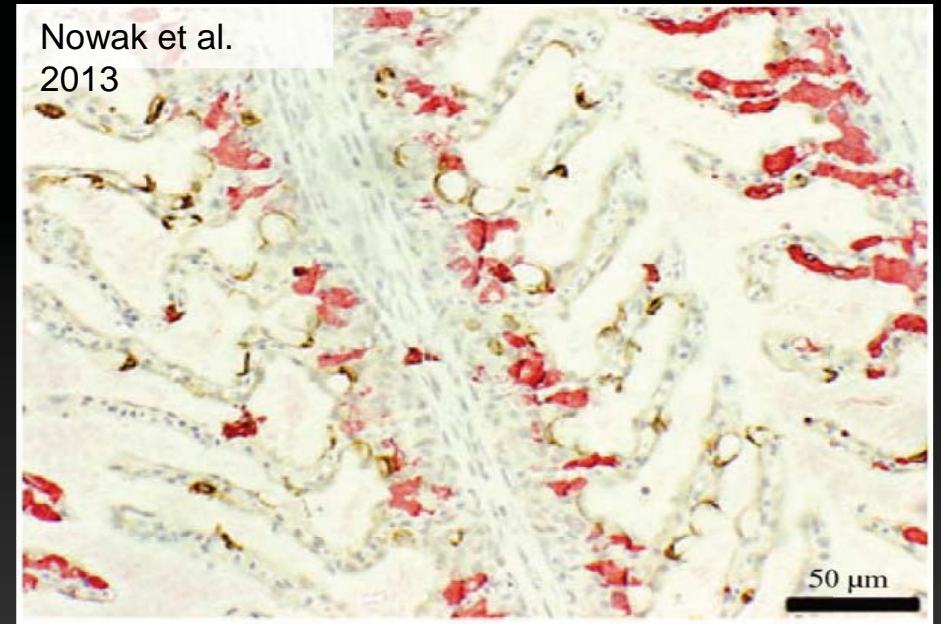
Red = over expressed

Green = under expressed

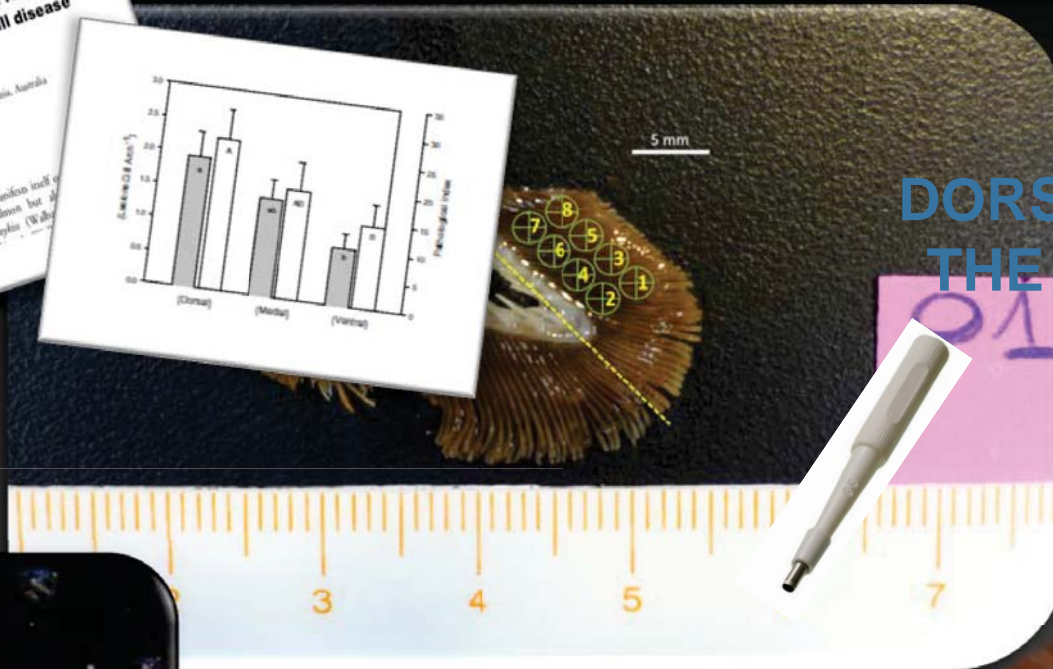
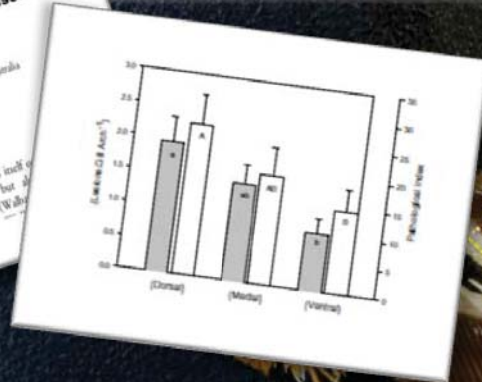
In AGD-samples

Proteins Identified Highlights

- asAG-2:
 - Increased in AGD-affected gill mucus (in this study)
 - highly expressed in cells within AGD-lesions
- Complement C3
 - Increased in AGD mucus samples (this study)
 - Associated with IL-1 β signalling
 - Recently associated with IgT in response to skin parasites in rainbow trout (Gomez et al. 2013)



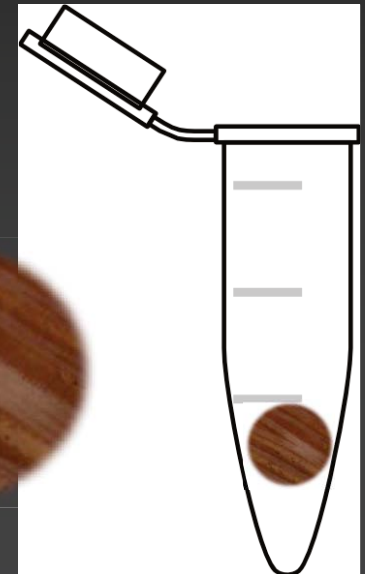
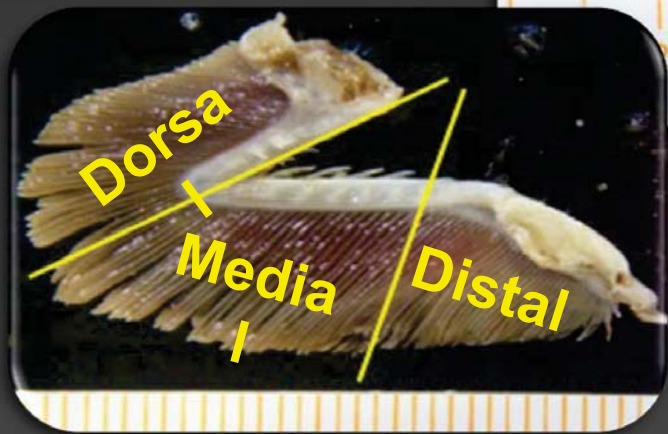
Gene expression in AGD



SELECTED AREA



DORSAL REGION OF THE 2ND LEFT GILL ARCH

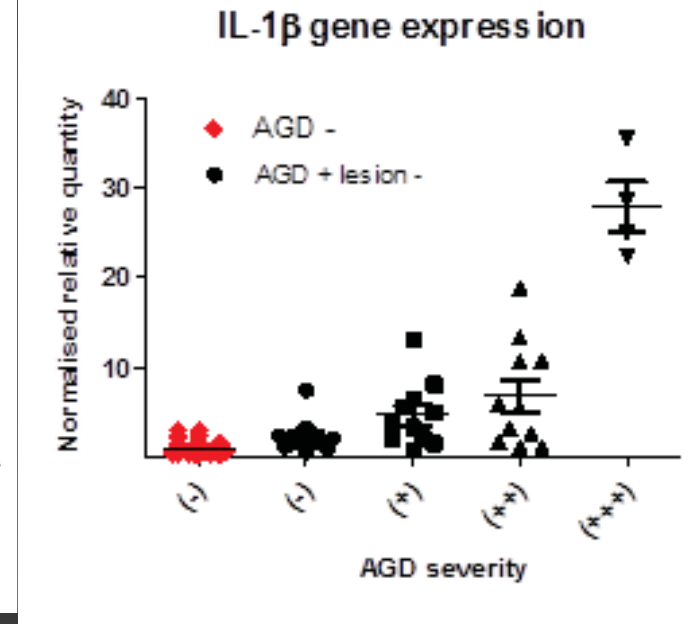
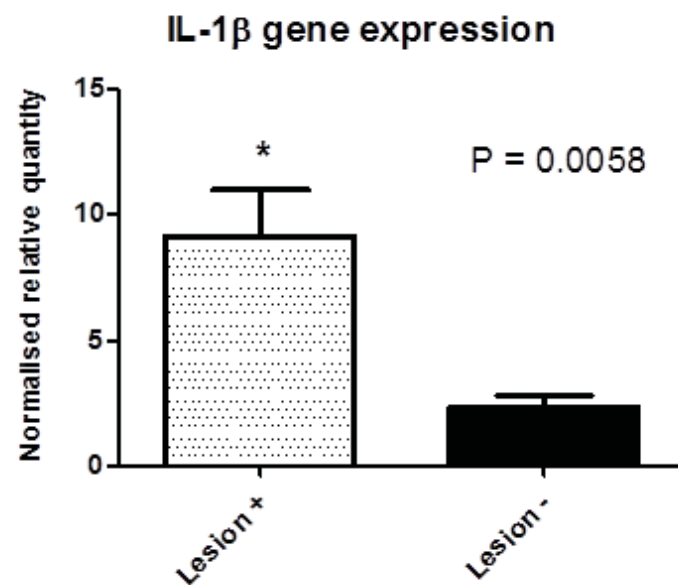
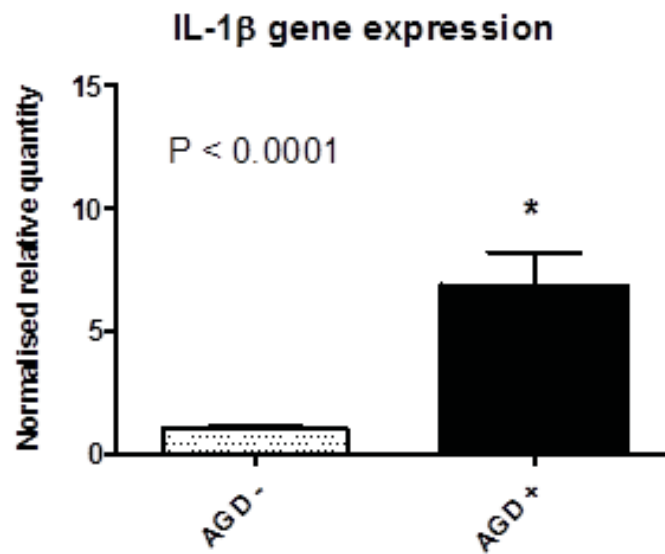


Assessment of AGD severity

- **AGD severity (-):** no lesion
- **AGD severity (+):** light lesions, 1-3 affected gill filaments and lesion area 10-30%
- **AGD severity (++):** 3-5 affected gill filaments and lesion area 30-50%
- **AGD severity (+++):** >5 affected gill filaments and lesion area >



Gill IL-1 β expression day 10 p.i.



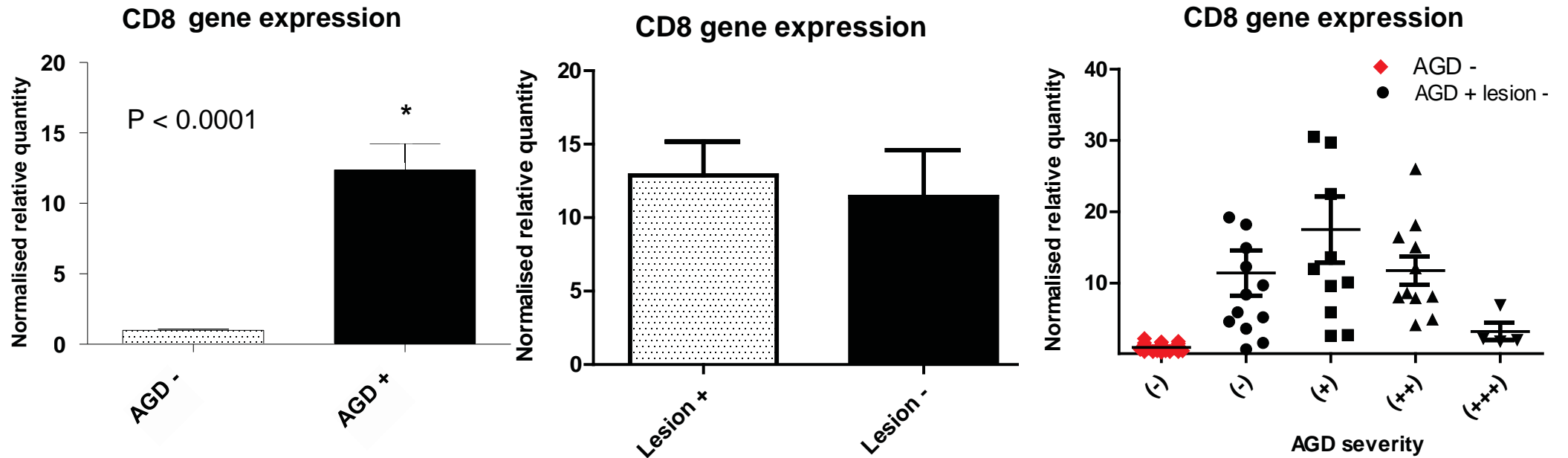
Fold
Change

AGD -	AGD +
1.0	6.9

Lesion +	Lesion -
9.1	2.3

C(-)	-	+	++	+++
1.0	2.3	4.6	6.8	27.8

Gill CD8 expression day 10 p.i.



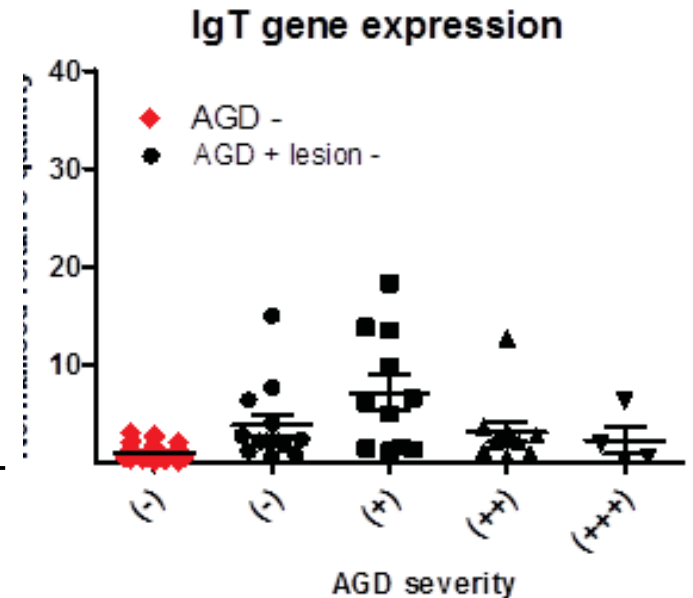
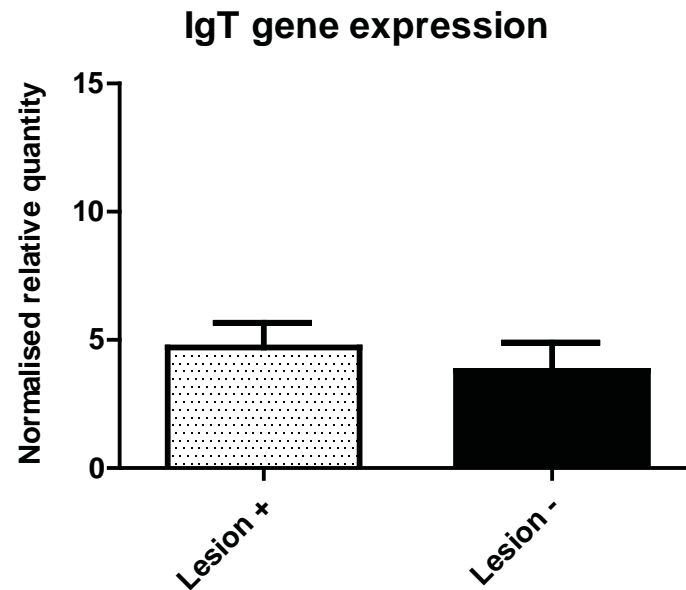
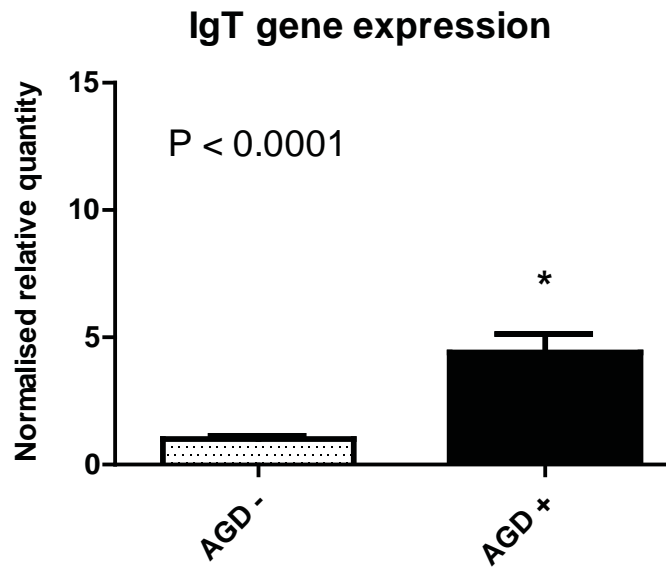
Fold
Change

AGD -	AGD +
1.0	12.4

Lesion +	Lesion -
12.8	11.4

C(-)	-	+	++	+++
1.0	11.4	17.5	11.7	3.2
	4	5	7	

Gill IgT expression day 10 p.i.



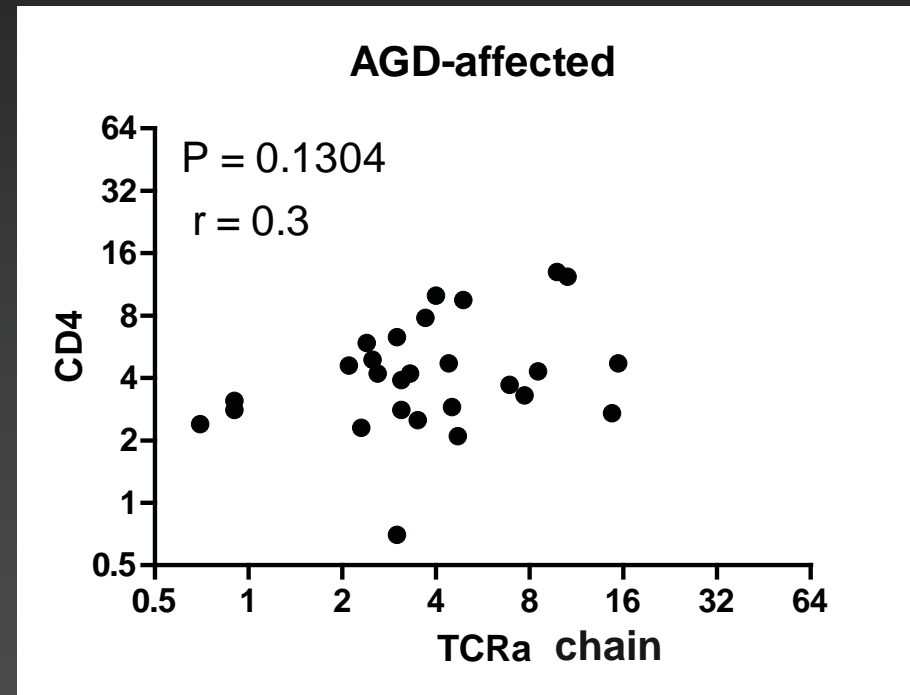
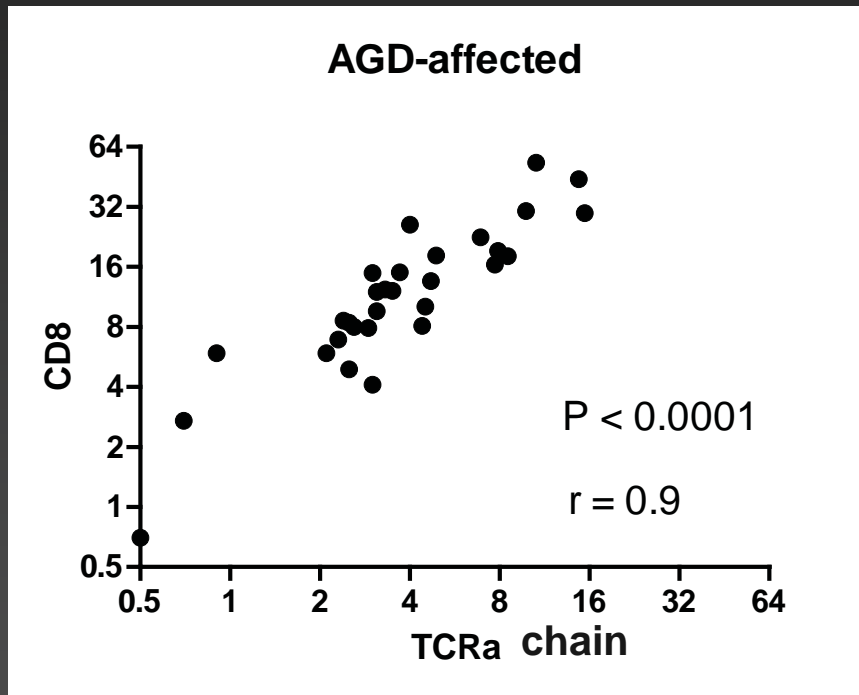
Fold Change

AGD -	AGD +
1.0	4.4

Lesion +	Lesion -
4.7	3.7

C(-)	-	+	++	+++
1.0	3.7	7.1	3.1	2.2

Correlation CD8, CD4 and TCR- α chain



Trial: repeated exposures to *N. perurans*



Analysis: immune gene expression (q RT-PCR)



Results and discussion:

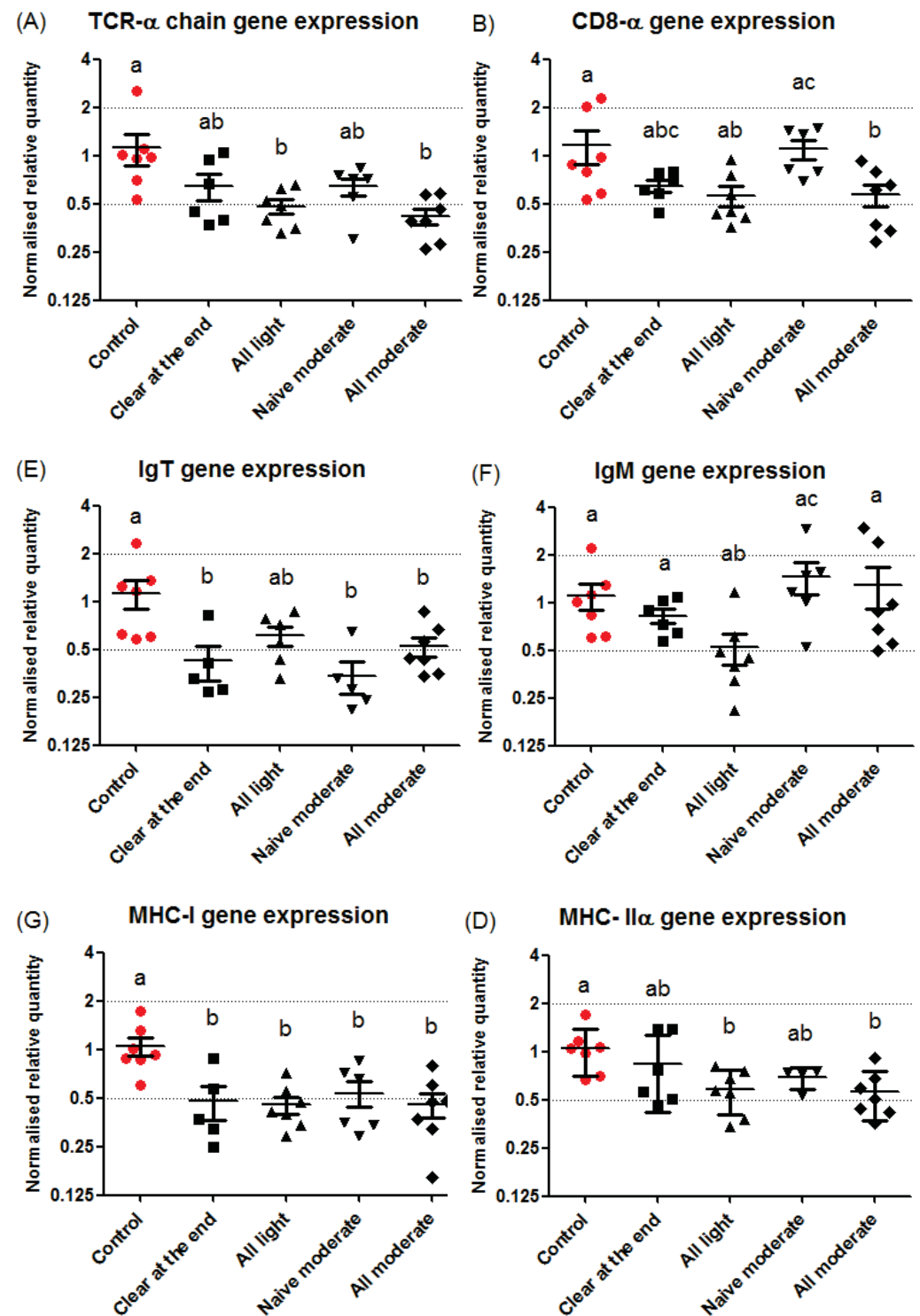
B-cells (IgM, IgT)

T-cells (TCR, CD8)

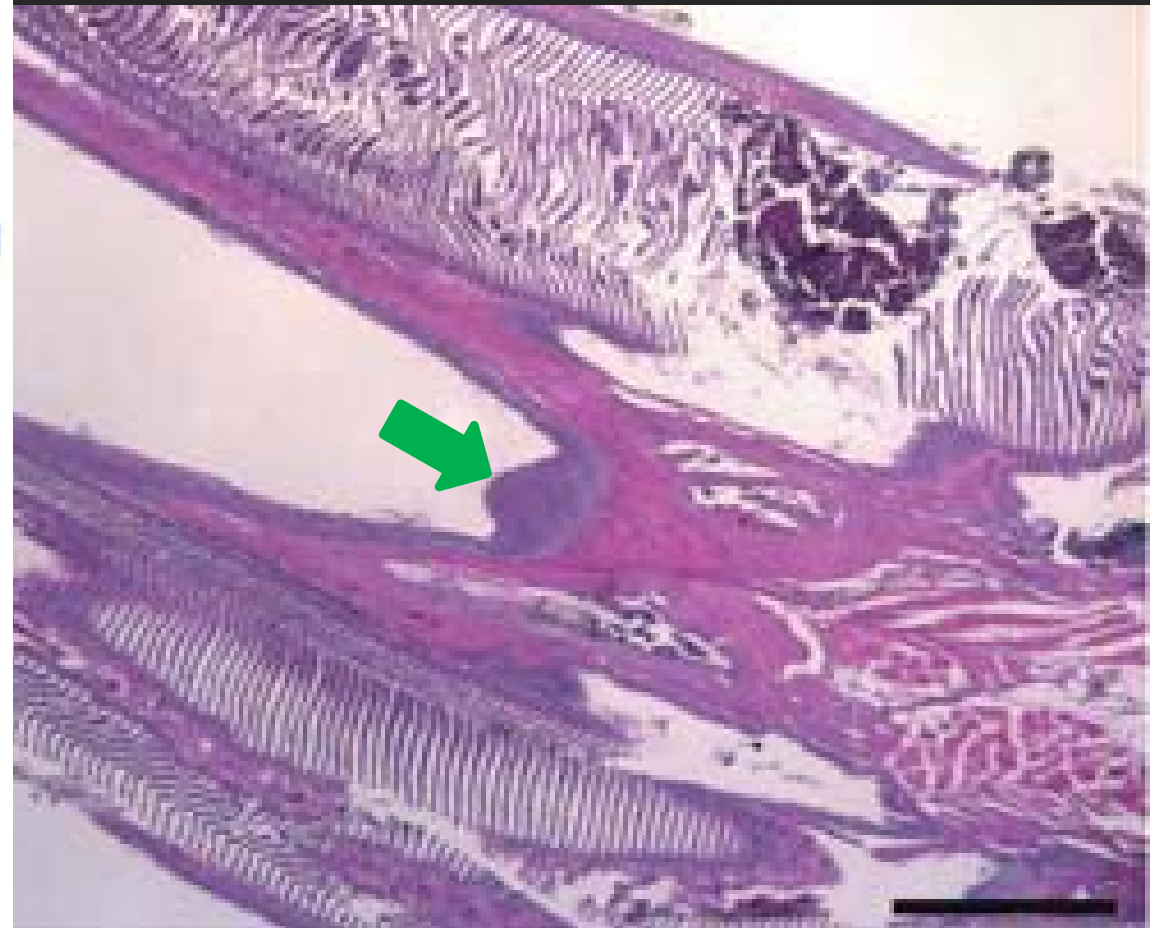
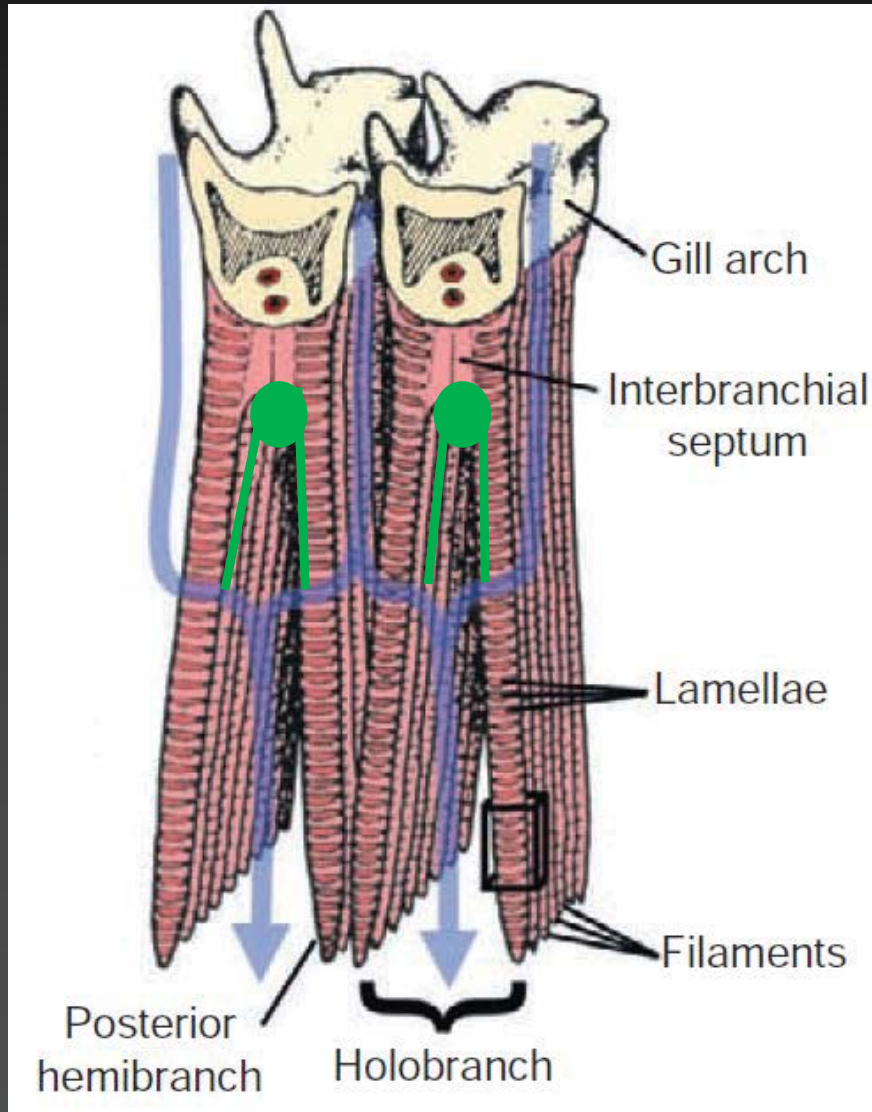
APC (MHCII, CD4)



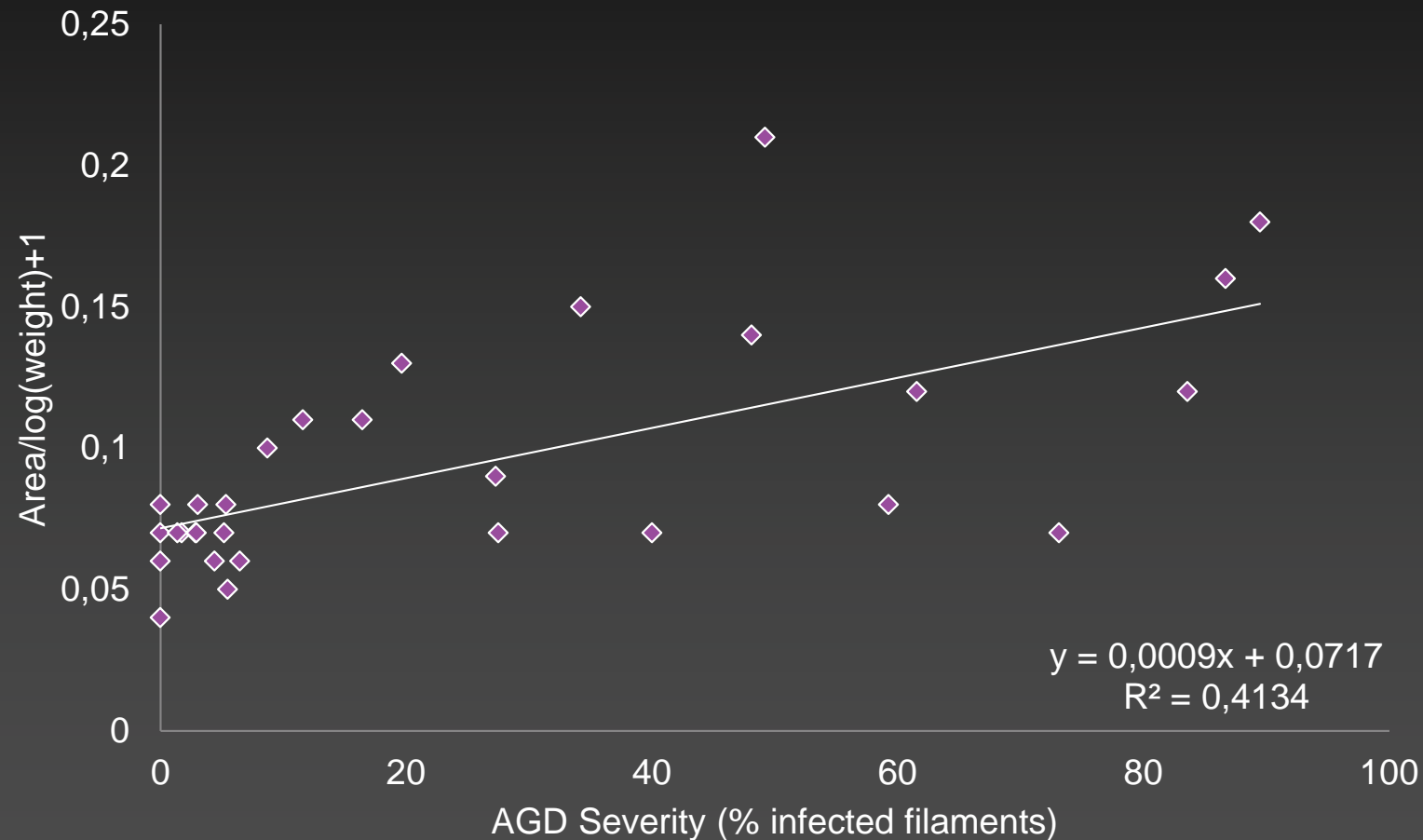
Decrease of host immune response in infection site (gill)

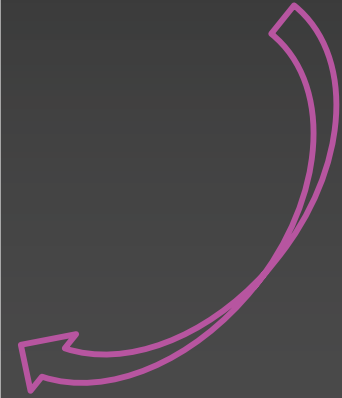
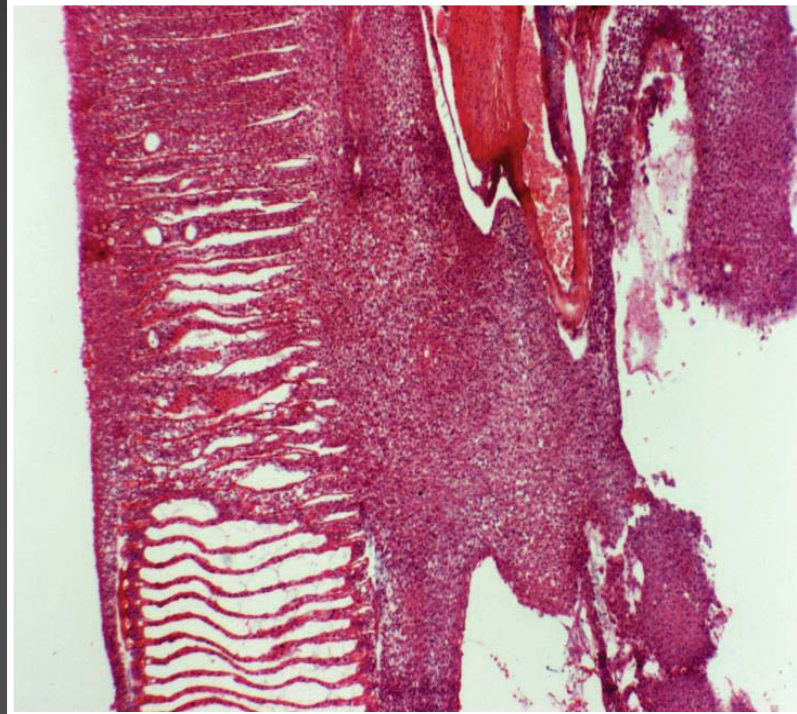
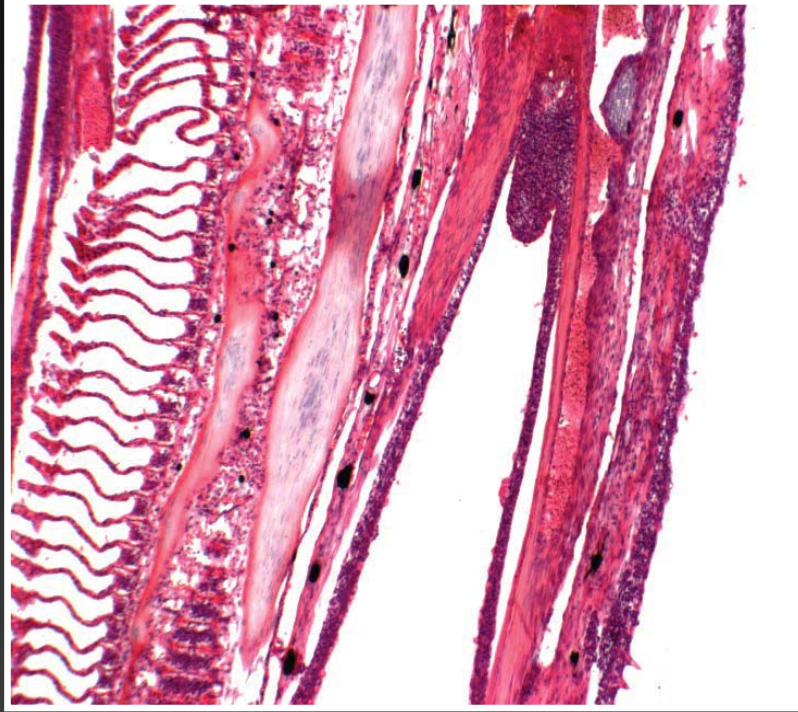


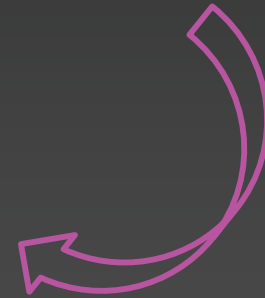
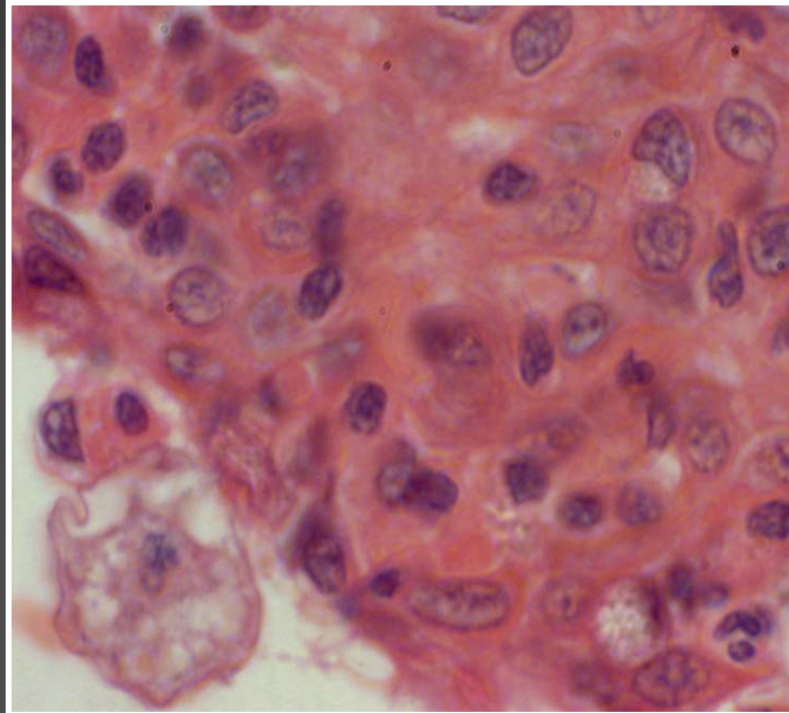
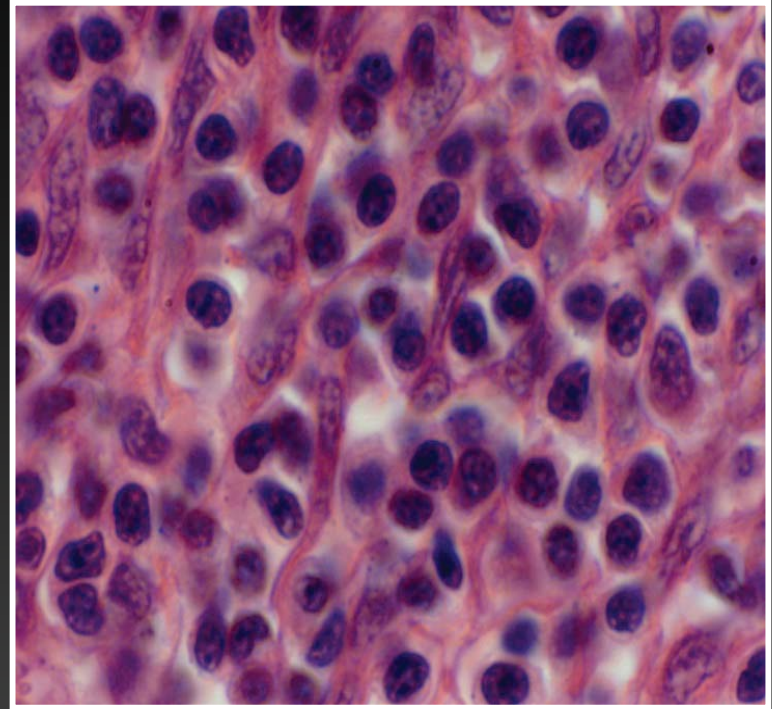
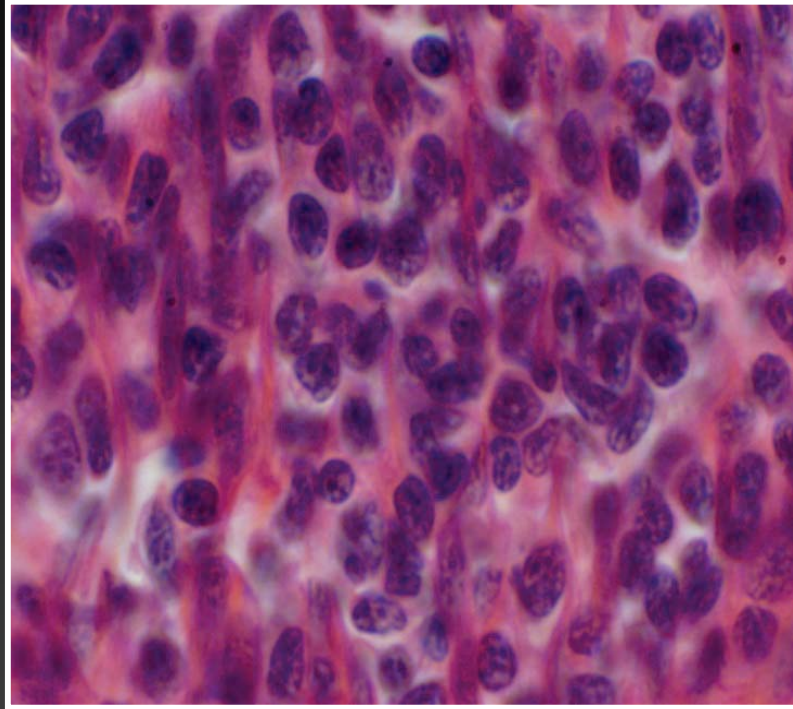
Interbranchial lymphoid tissue (ILT)



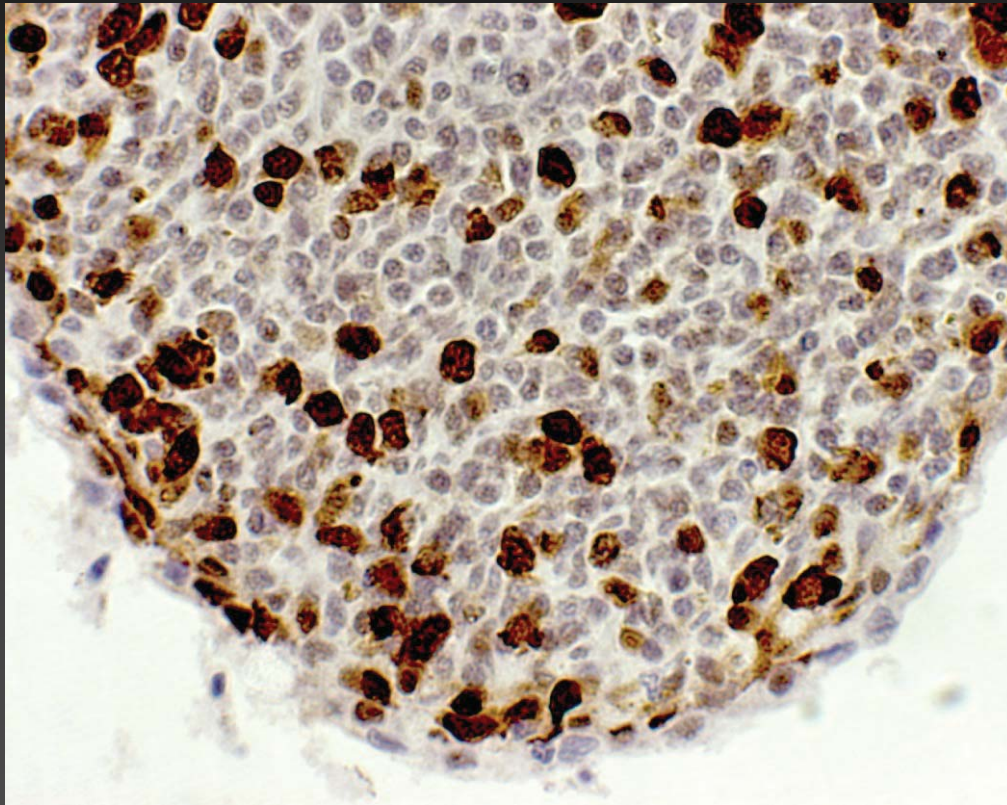
Relationship between ILT area and AGD severity



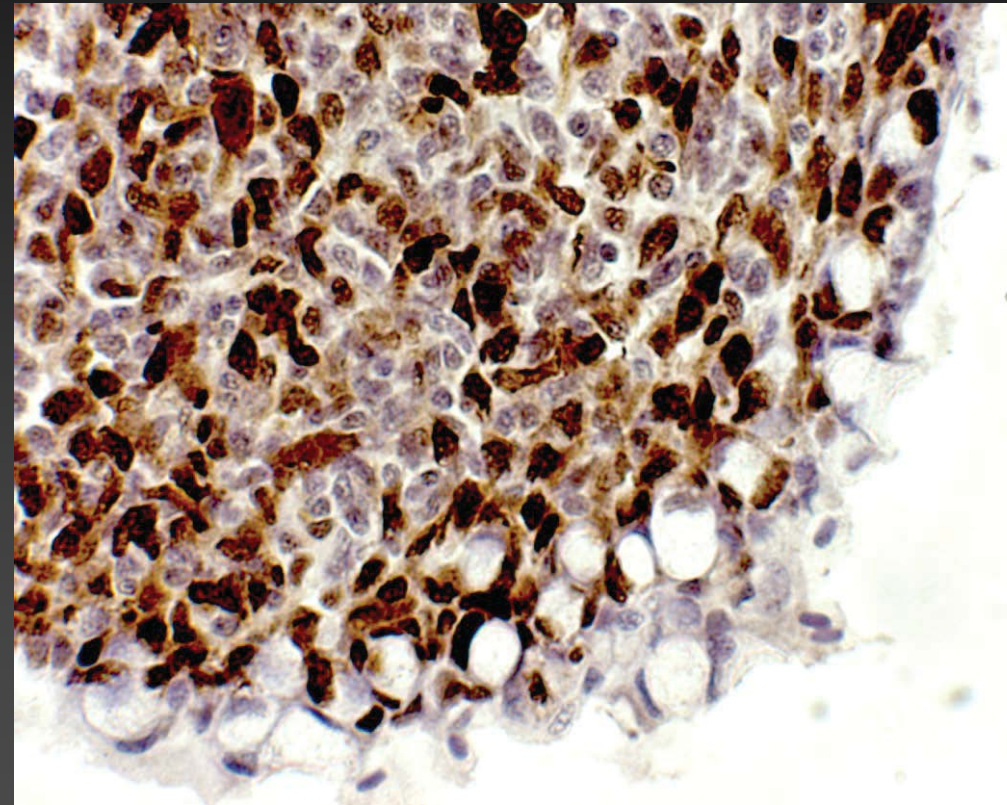




Cell proliferation



Control fish - 14 days post infection



Infected fish - 14 days post infection

Current AGD research

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- Treatments
- Susceptibility of other species
- Immune response

Acknowledgements

- FRDC, Seafood CRC
- Salmon industry
- Victoria Valdenegro, Stewart Dick, Dr Andrew Bridle, Dr Phil Crosbie, Dr Mark Adams, Dr Melanie Leef Mark Polinski, Karine Cadoret , Ylenia Pennacchi, Catarina de Carmo Norte dos Santos, Thomas Hill, Paul Li, Danielle Devonport (University of Tasmania)
- Prof Ben Koop (University of Victoria, Canada)
- Prof Chris Secombes (University of Aberdeen, Scotland)
- Prof Erling Koppang (Norwegian Veterinary College, Norway)
- Dr Tim Dempster (University of Melbourne)