

What do we know about the immune system of meagre and amberjack?





Project aims

Meagre (*Argyrosomus regius*)

1) Gene discovery

Develop tools for monitoring gene expression of Key immune molecules

2) Antibody development

Develop antibodies against IgM and IgT

3) Tissue stimulation

Identify methods of inducing and monitoring immune gene expression

4) Ontogeny study

Use the designed tools to interrogate the development of the immune system from juveniles to adult fish

5) Vaccine challenge

Monitor the immune response to potential pathogens such as *Vibrio anguillarum* (vaccine provided by IRTA)

Meagre : Gene Discovery

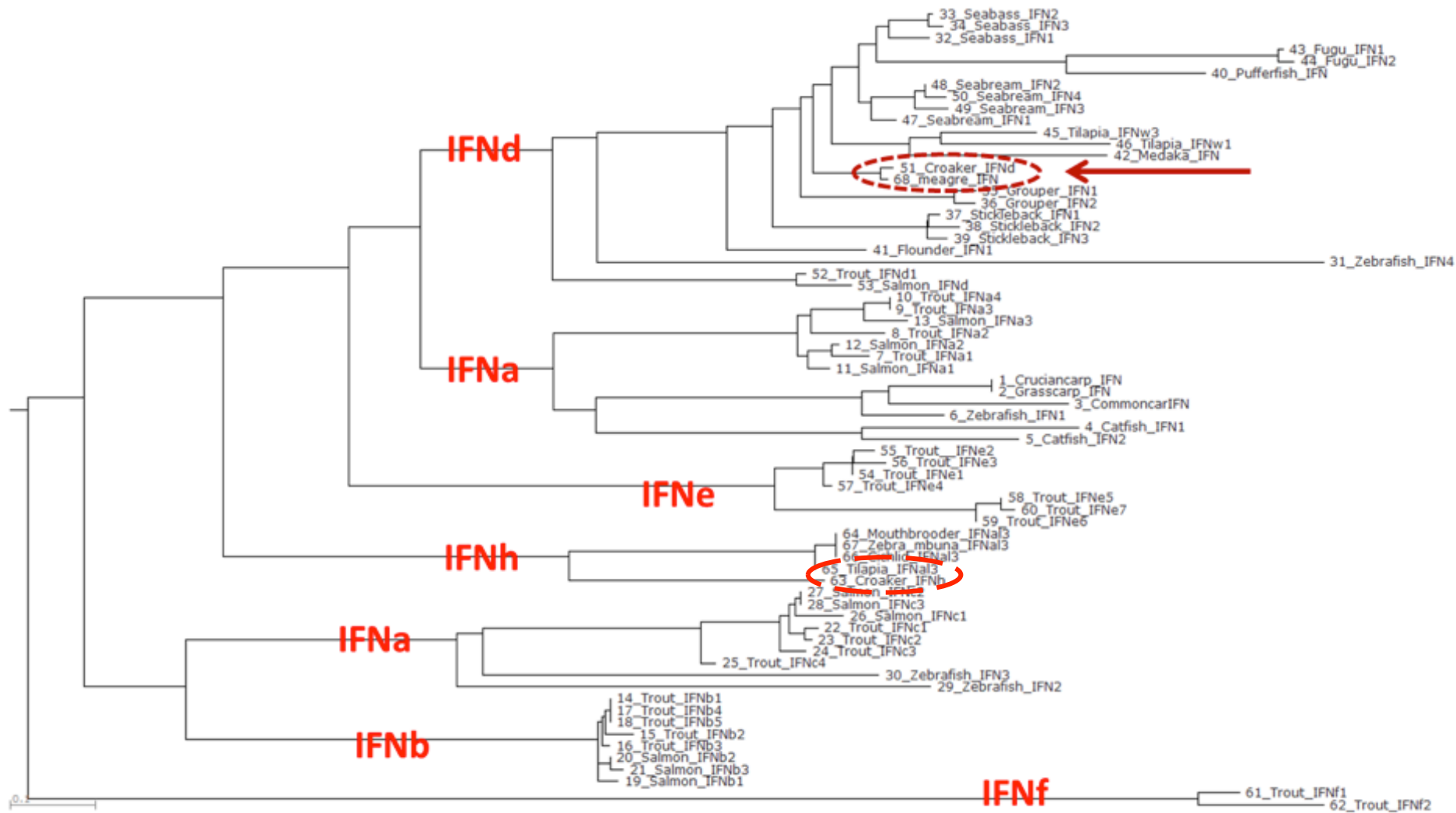
Adaptive Immune Genes (Aberdeen)

CYTOKINES	IL-1B	ANTI-MICROBIAL PEPTIDES	β - DEFENCIN
	IL-4/13		HEPCIDIN
	IL-10		PISCIDIN
	IL17AF	OSTEOCLAST / OSTEOBLAST	CATHESPIN K
	IL-22		V-ATPASE
	TNFa2		TRAP
	IFN1	HOUSEKEEPING GENES	EF1 α
	IFN γ		β -ACTIN
IgM	HPRT		
IMMUNOGLOBULIN RELATED	IgD		
	IgT		
	RAG1		
	TCRB		

Total of 33 genes !

Innate Immune gene (IRTA)

ACTIVE DEFENCE PROTIENS	Met
	Lysozyme
	C3
	Mx
	TNFa1
RECEPTERS AND SGNALING MOLECULES	NOD2
	NOD3
	COX2
	MYD88
HOUSEKEEPING GENES	EF1 α
	GADPH
	18s

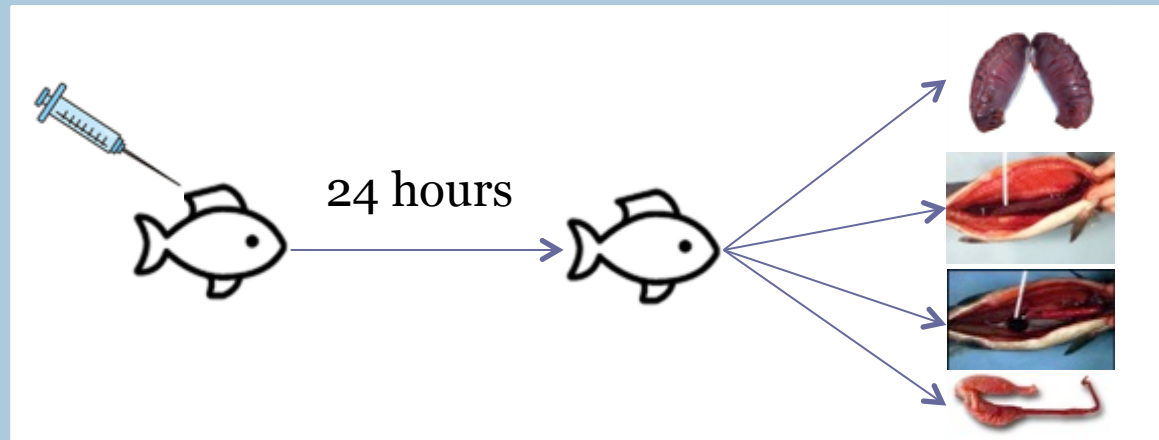


Meagre Tissue Stimulation Setup

In Vivo

Ip Injection of fish

- PBS
- Poly I:C
- LPS
- β - Glucan

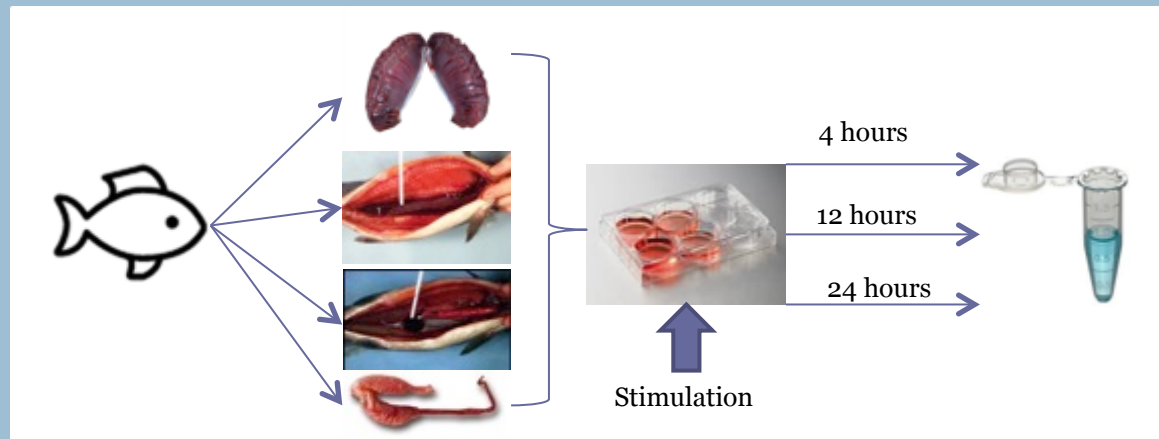


In Vitro

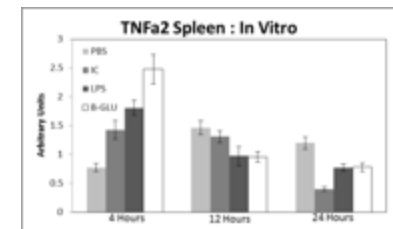
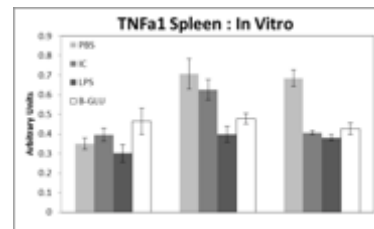
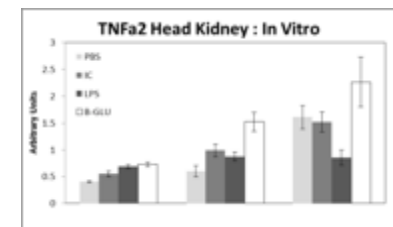
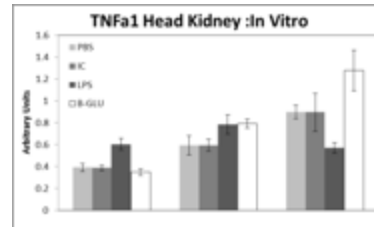
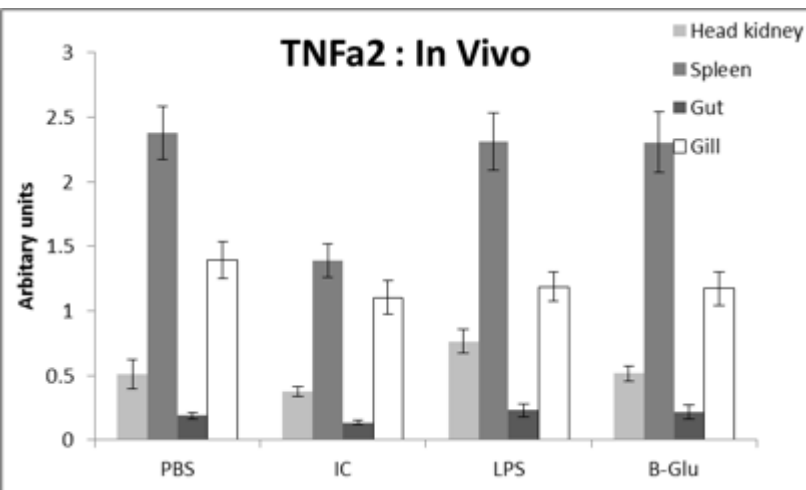
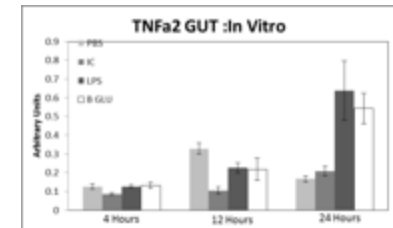
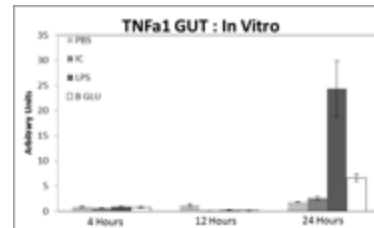
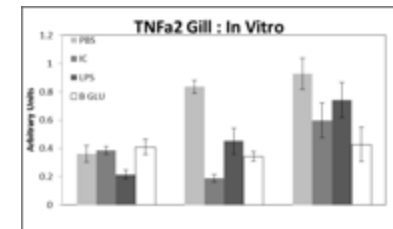
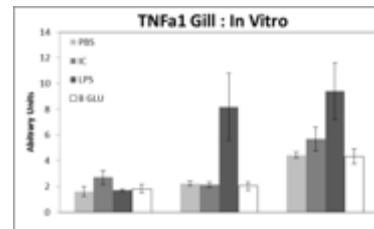
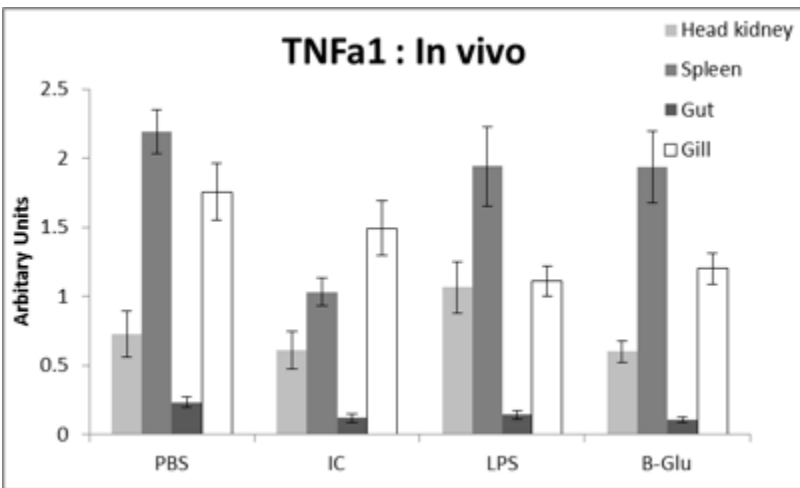
Primary cell culture stimulated by:

- PBS
- Poly I:C
- LPS
- β - Glucan

Samples taken after 4, 12 and 24 hours

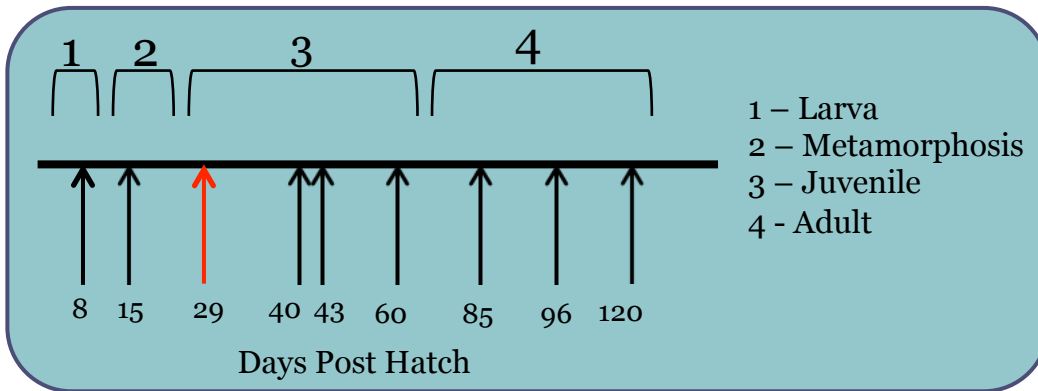


Meagre Tissue Stimulation Results



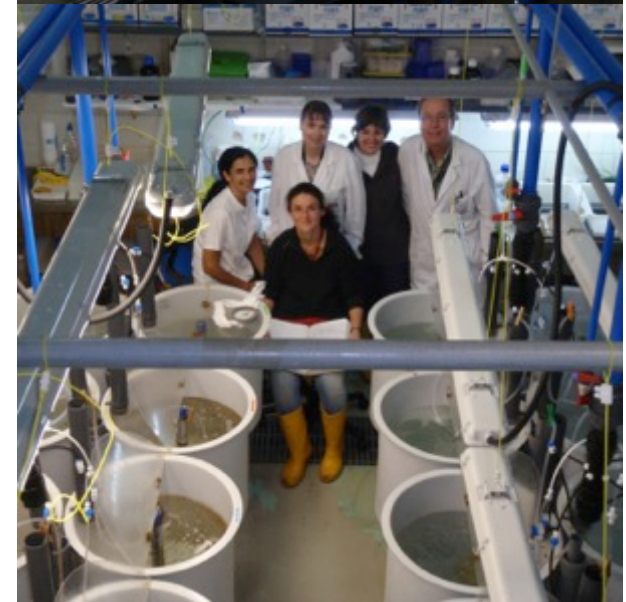
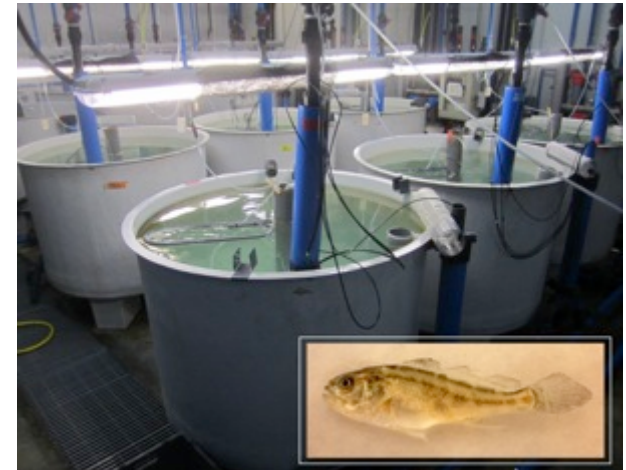
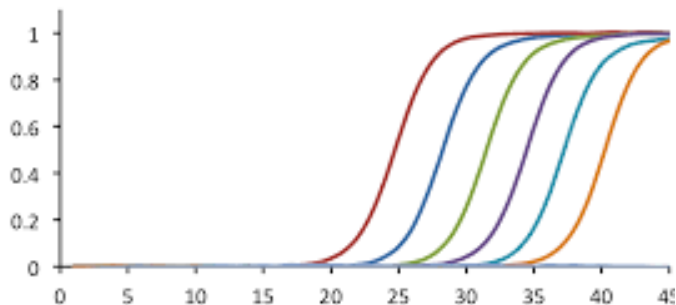
Meagre Ontogeny study

1) Collect meagre samples from IRTA

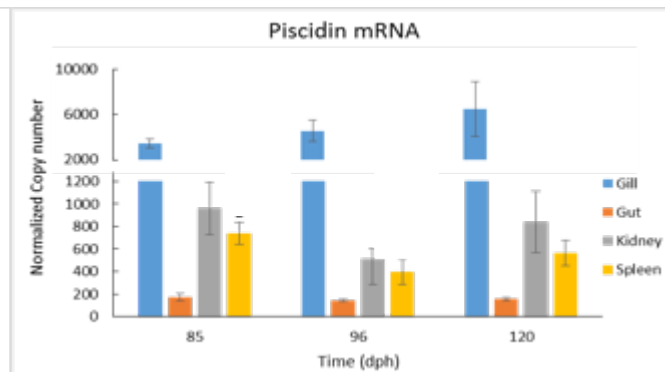
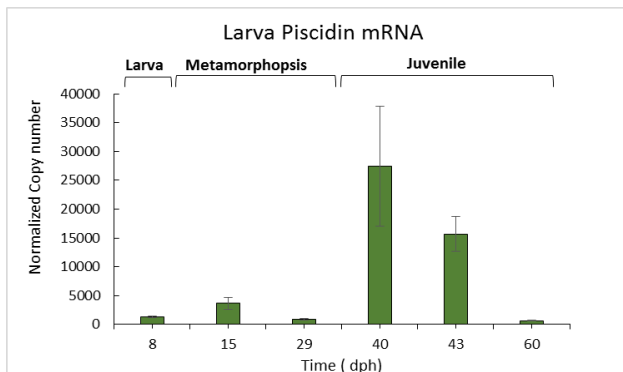
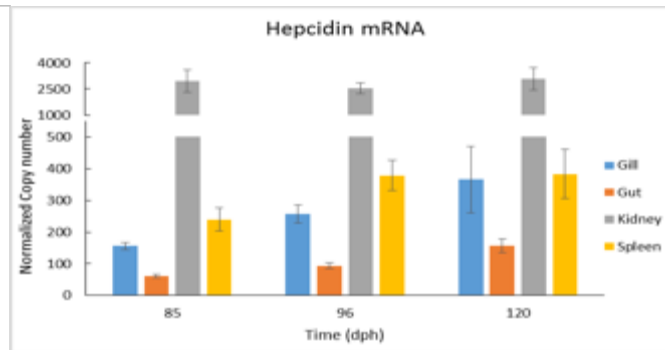
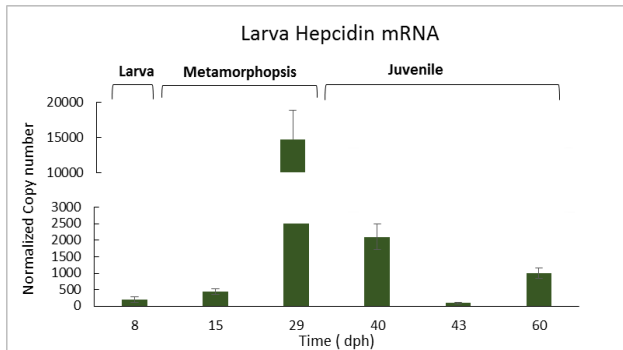
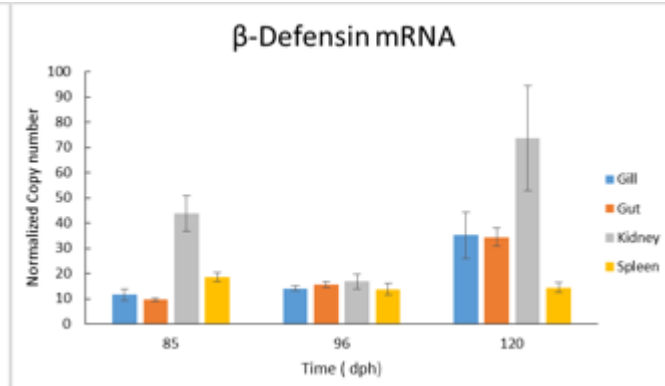
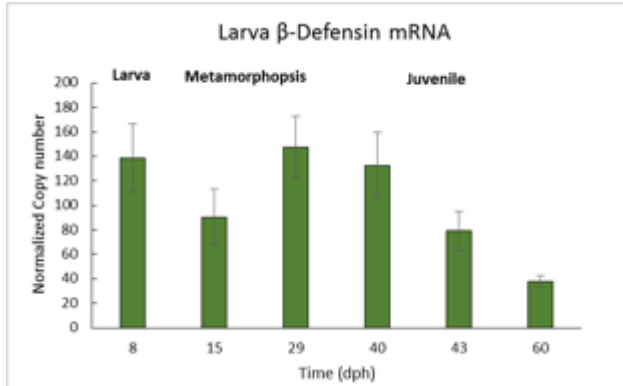


2) RNA extraction from whole larva (8-60 dph) and immune tissues (85-120 dph) followed by CDNA synthesis

3) Perform QPCR



Meagre ontogeny results : AMPs

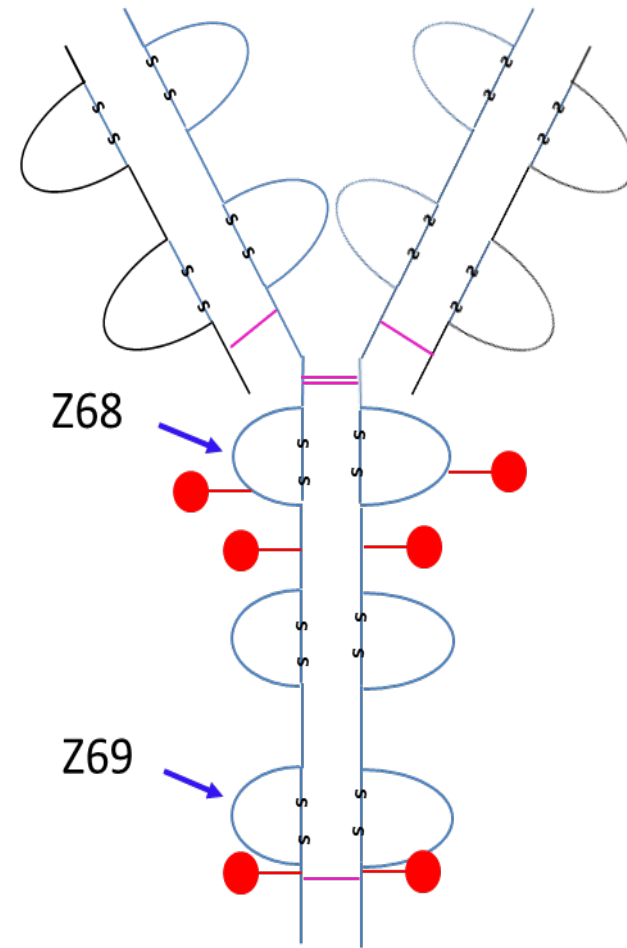


 Graphs
 provided by
 Cindy
 Campoverde

Meagre Antibody Development

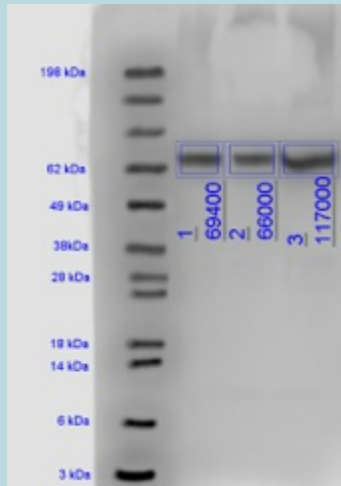
Peptide needs to be :

- Immunogenic
- Hydrophilic
- Accessible
- Unique to Target



Meagre Antibody Development

Meagre Anti IgM (Z69)



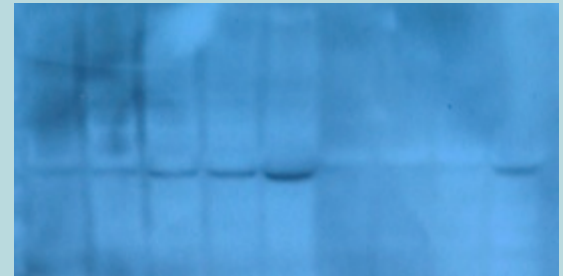
Lane 1 Ladder
Lane 2 Serum 1:30
Lane 3 Serum 1:15
Lane 4 serum 1:10

Meagre Anti IgM (Z69) and Anti IgT (Z55)



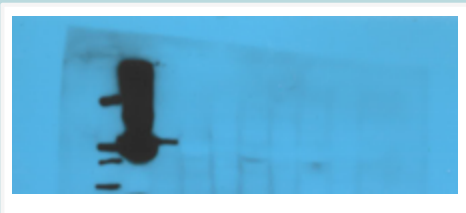
Lane 1 serum 1:15 anti IgM
Lane 2 serum 1:15 anti IgT

Meagre anti IgT (Z55) Multiple species



Lane 1 serum 1:30 Meagre
Lane 2 serum 1:30 Lumpfish
Lane 3 serum 1:30 seabream
Lane 4 serum 1:15 seabream
Lane 5 serum 1:10 seabream
Lane 6 serum 1:30 Large Yellow Croaker
Lane 6 serum 1:15 Large Yellow Croaker
Lane 6 serum 1:10 Large Yellow Croaker

Meagre anti IgM (Z69) Multiple species



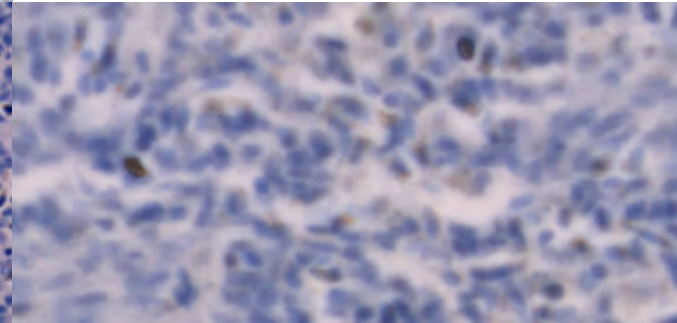
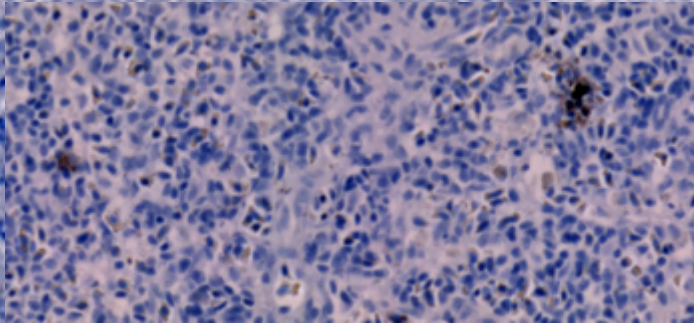
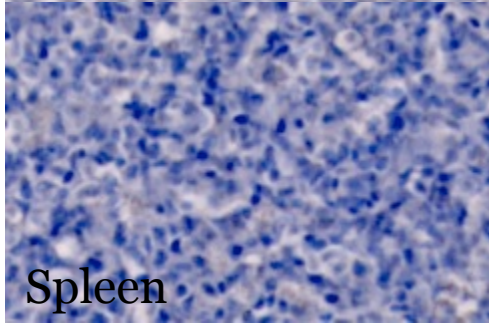
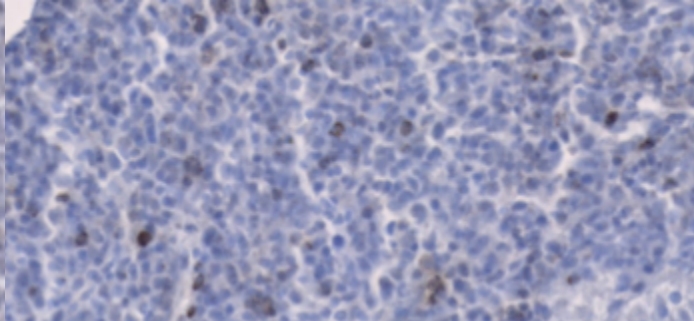
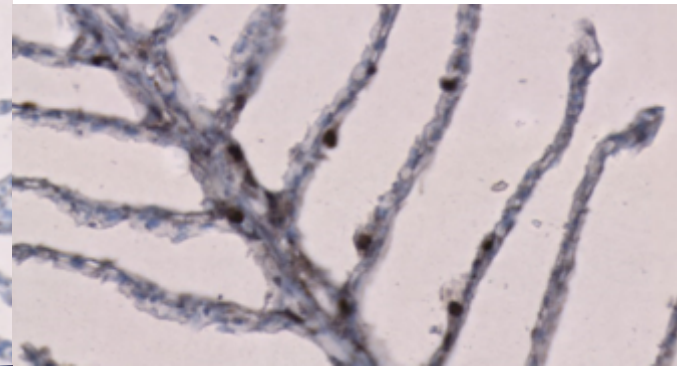
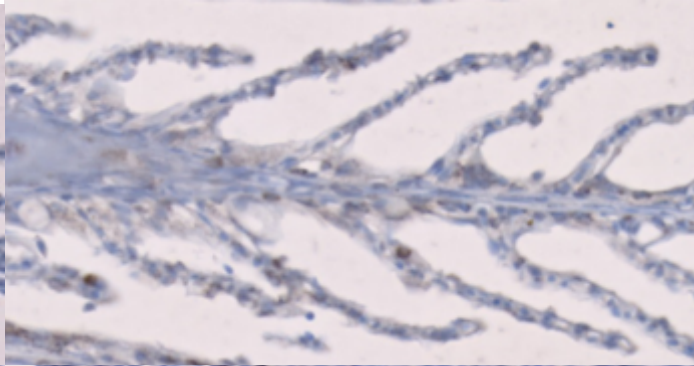
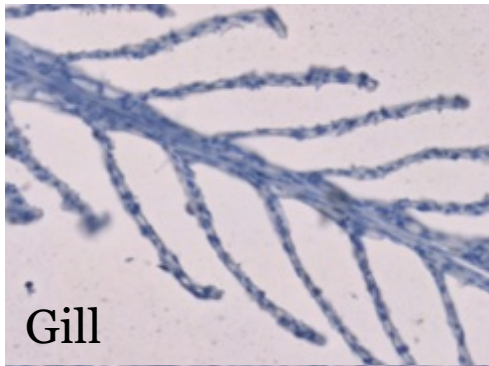
Lane 1 ladder
Lane 2 serum 1:5 meagre
Lane 3 serum 1:5 lumpfish

Meagre Antibody Development

Control

IgM (Z69)

IgT (Z55)



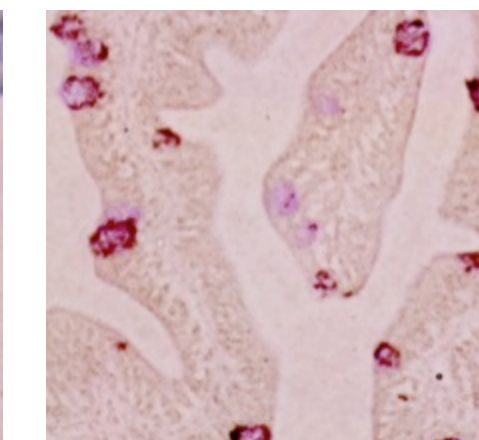
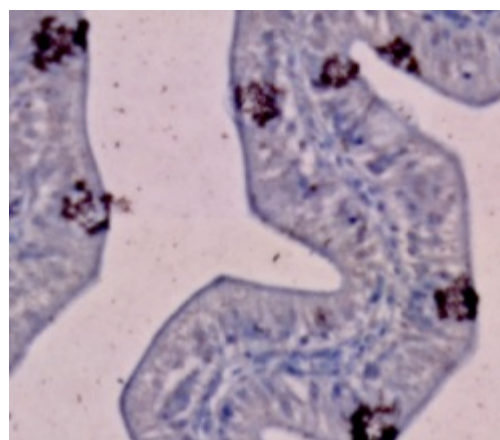
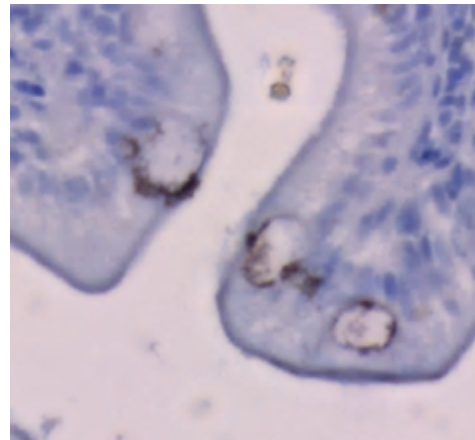
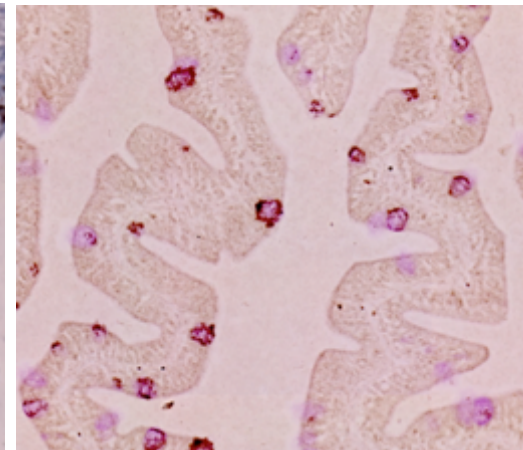
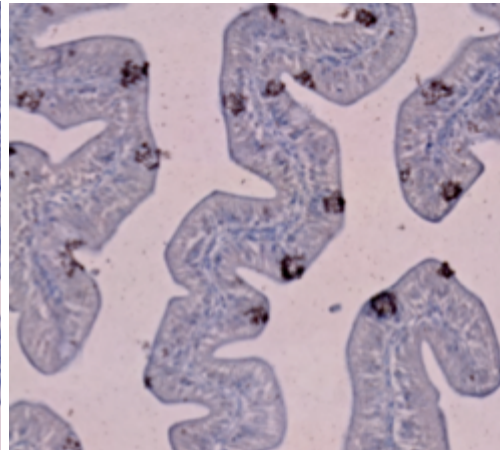
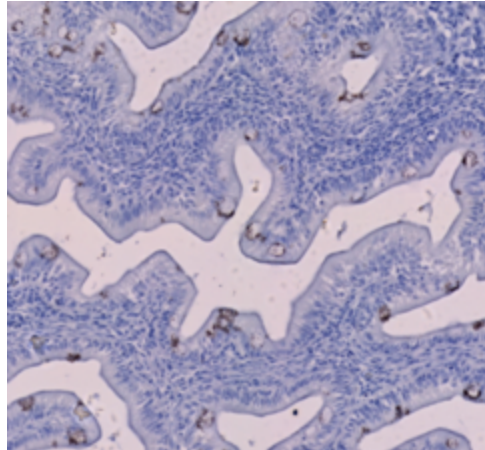
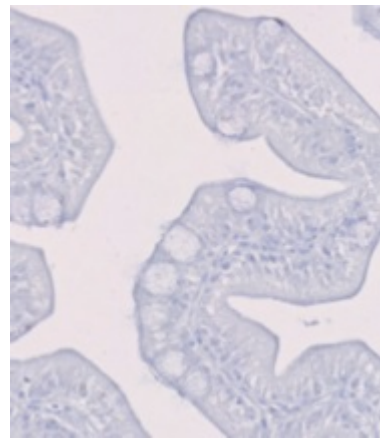
Meagre Antibody Development

Control

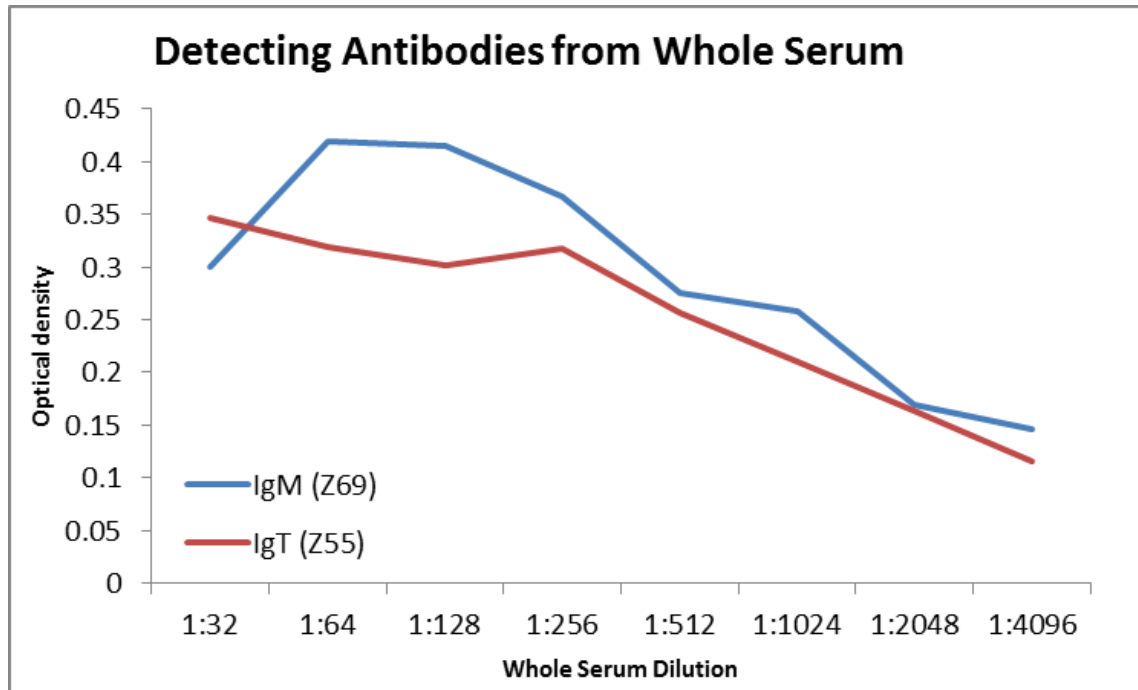
IgM (Z69)

IgT (Z 55)

IgT (Z55) / PAS



Meagre Antibody Development



- Whole Serum used was from naive meagre
- Able to detect IgM and IGT from whole serum when whole serum is diluted 1:16 – 1:512



Project aims
Greater amberjack (*Seriloa dumerili*)

1) Gene discovery

Develop tools for monitoring gene expression key genes in MUCOSAL immunity

2) Tissue stimulation

Identify methods of inducing mucosal immune gene expression with an emphasis on the gills as a model of mucosal immunity

3) Diet and immunity

Monitor the impact of dietary regimes on immune gene expression

4) Mucus immune molecules

Characterise the key molecules in mucus defences

Gene Discovery

Amberjack genes Discovered

Cytokines	IL1B	Immunoglobulin Related	RAG2
	IL8		IgM
	IL10		IgT
	IL17AF	Anti viral	Mx
	IL17D		INOS
	IL22		IFN1
	TNFa		IFN γ
Anti – microbial Peptides	β -DEFENCIN	Housekeeping genes	EF1a
	HEPCIDIN		β -ACTIN
	PISCIDIN		

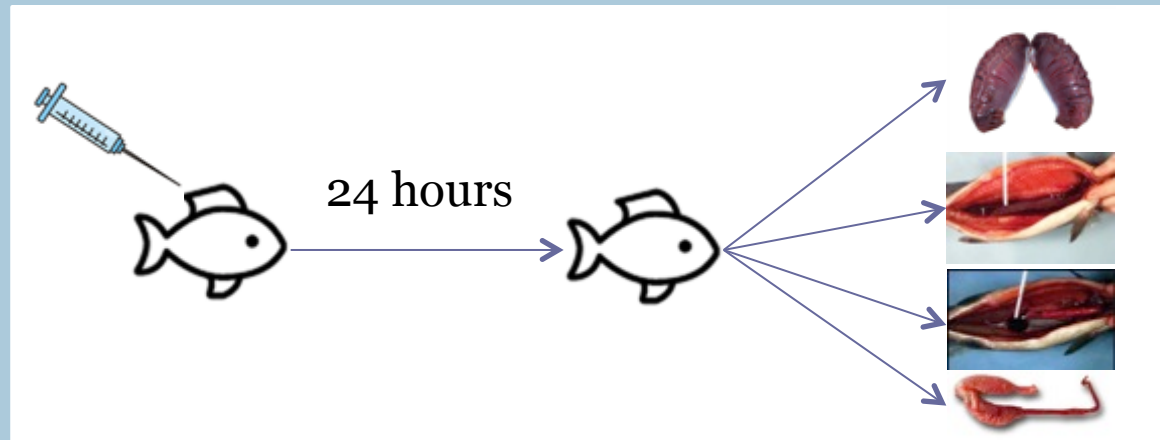
19 Genes Discovered !

Amberjack Tissue Stimulation Setup

In Vivo

Ip Injection of fish

- PBS
- Poly I:C
- LPS
- Flagellin

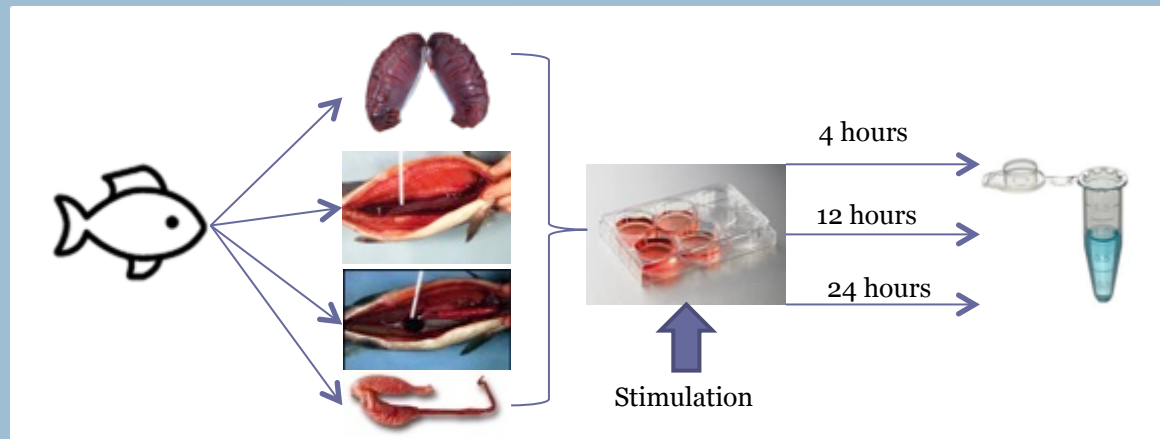


In Vitro

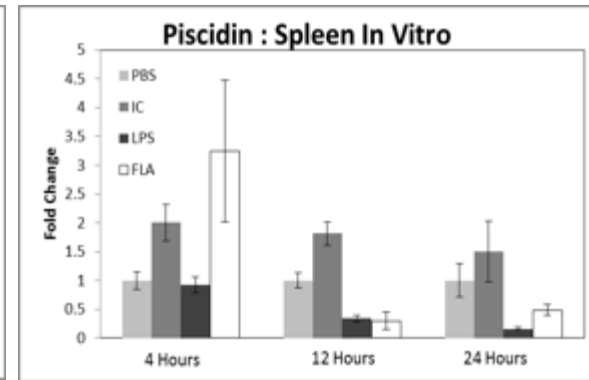
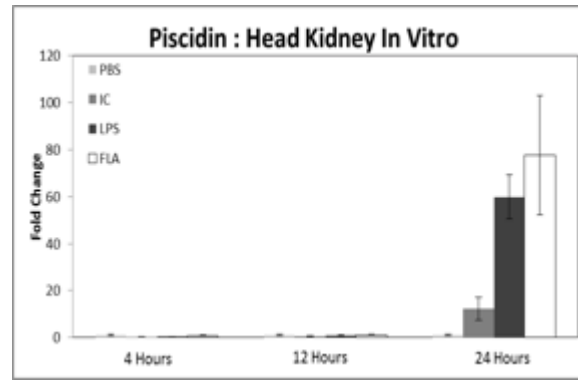
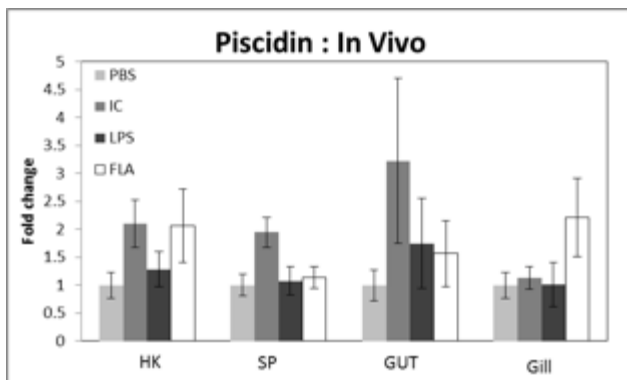
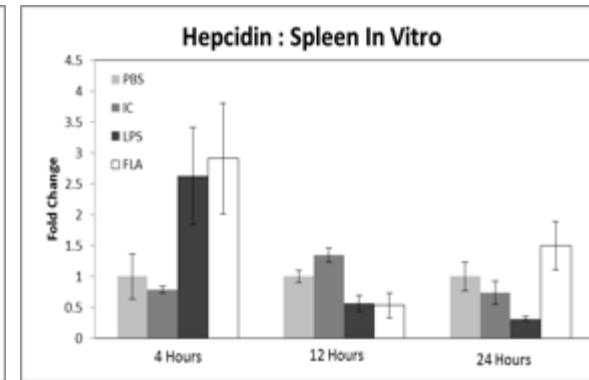
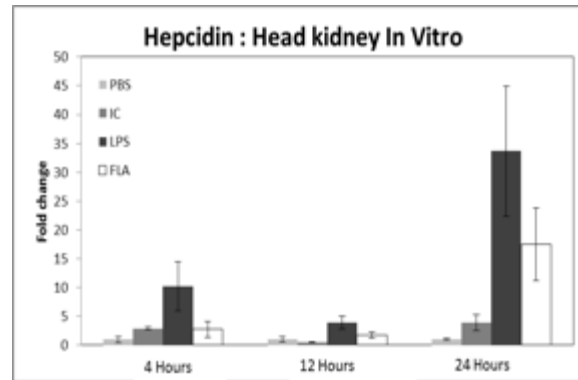
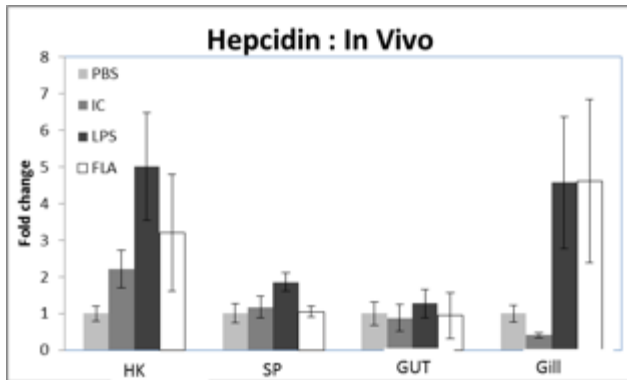
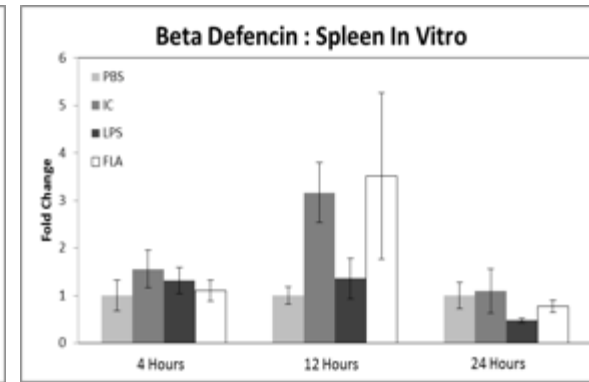
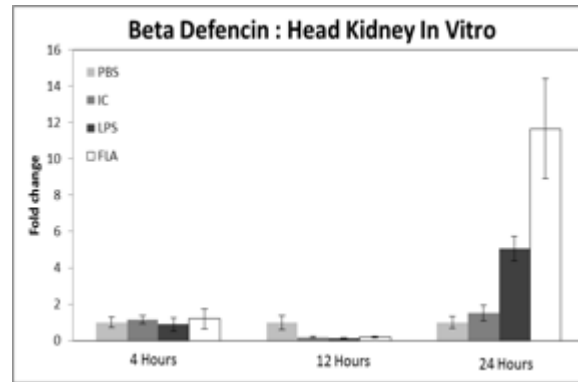
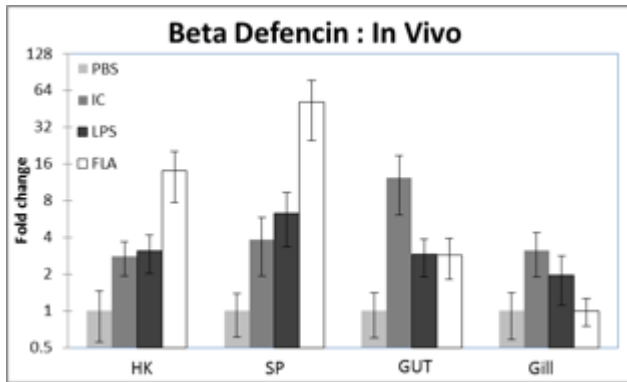
Primary cell culture stimulated by:

- PBS
- Poly I:C
- LPS
- Flagellin

Samples taken after 4, 12 and 24 hours



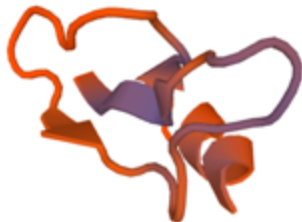
Amberjack Tissue Stimulation Results



Amberjack AMPS

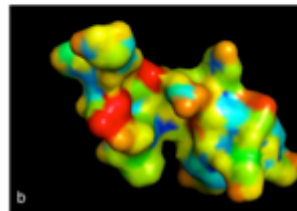
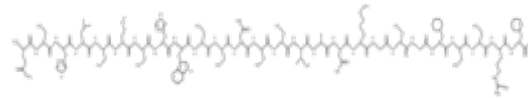
β – Defencin

Length: 42
Mass: 4492.1456
Isoelectric point (pI): 8.00
Net charge: +2
Hydrophobicity: +11.12 Kcal * mol⁻¹
Extinction coefficient¹: 5875 M⁻¹ * cm⁻¹
Extinction coefficient²: 5500 M⁻¹ * cm⁻¹



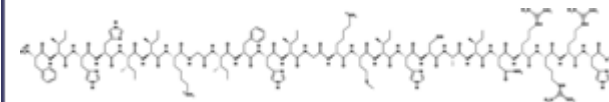
Hepcidin

Length: 26
Mass: 2941.0712
Isoelectric point (pI): 7.83
Net charge: +2
Hydrophobicity: +16.02 Kcal * mol⁻¹
Extinction coefficient¹: 6000 M⁻¹ * cm⁻¹
Extinction coefficient²: 5500 M⁻¹ * cm⁻¹



Piscidin

Length: 25
Mass: 3030.7145
Isoelectric point (pI): 12.79
Net charge: +5
Hydrophobicity: +22.76 Kcal * mol⁻¹
Extinction coefficient¹: 0 M⁻¹ * cm⁻¹
Extinction coefficient²: 0 M⁻¹ * cm⁻¹

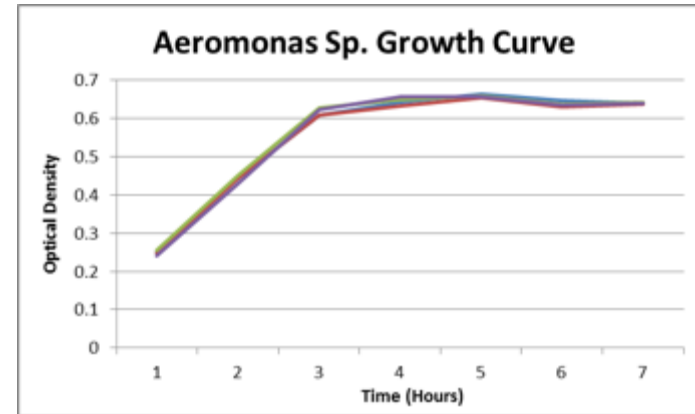


Amberjack AMPS Current work

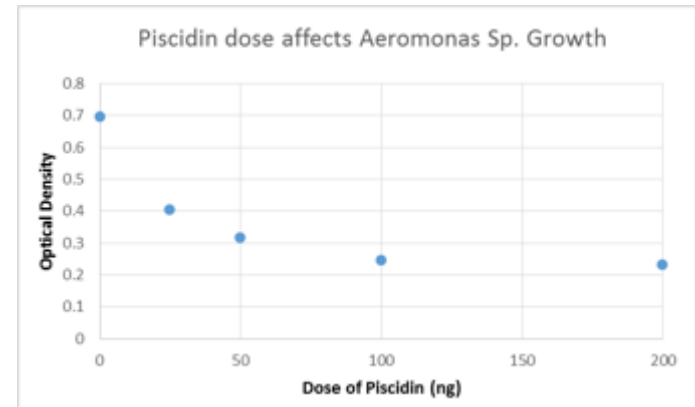
1) Synthesise Amberjack Piscidin active peptide



2) Determine bacterial exponential growth phase

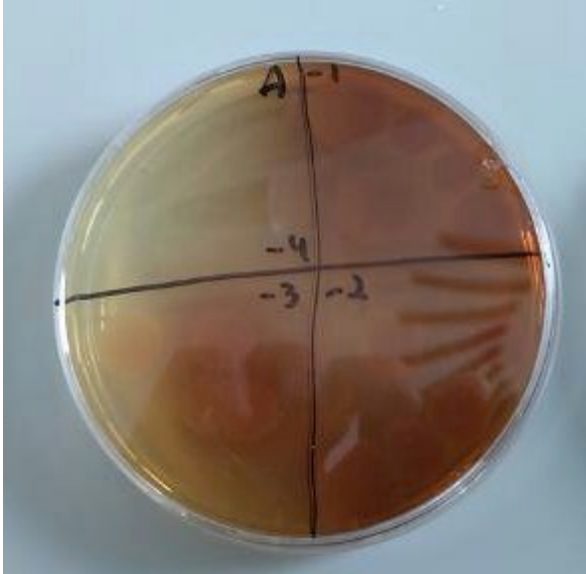


3) Determine the efficacy of active peptide by subjecting bacteria to a dose gradient (MIC = 12.5 ng/ml)



Amberjack AMPS Current work

4) Test Bacterial cell viability after Piscidin interaction



Future work

Meagre

Vaccine Trial (IRTA)

Short term effects

- Measure gene expression using QPCR (current work)

(long term effects)

- Measure specific antibody response, serum and mucus (current work)
- Measure Gene expression using QPCR

Antibodies

- Develop a 2nd anti IgT Mab and 2nd anti IgM Mab and potentially anti IgD Mab (current work)
- In situ hybridisation to determine Source of antibodies in the gut (current work)

Gene discovery

- Discover sequence for all type 1 interferons IFN α , IFN δ and IFN η (current work)

Amberjack

Diet Trial (FCPCT)

- Using QPCR determine the effect of diet on the immune response of amberjack

Anti – microbial Peptides

- Continue to screen bacterial and other pathogenic species to determine the efficacy of Piscidin (current work)

Acknowledgements



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Universidad de Las Palmas de Gran Canaria



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Dr Felix Acosta

Cindy Campoverde
(PhD Student)



Dr Pantelis Katharios

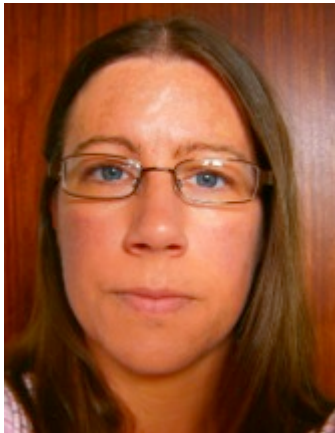
Acknowledgements



SCOTTISH
FISH IMMUNOLOGY
RESEARCH CENTRE



Laura Mollon M.Sc.



Dr Dawn Shewring



Dr Jason Holland



Dr Jun Zou
(Supervisor)



Prof Chris Secombes
(supervisor)

Questions ?