HEALTH ISSUES OF MEAGRE (*Argyrosomus regius*): prevention of infectious disease

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Co-funded by the Seventh Framework Programme of the European Union

Project DIVERSIFY

Within the Health Work Package for meagre there are specific topics one of which is *immune response and its development.* Investigations into the functional capacities of the immune system will provide support for:

1. understanding the immune capacities of fish during grow-out.
2. aid in management of disease outbreaks when they occur.

Meagre

Argyrosomus regiu

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Key Areas Of Immune Study

1) Development of tranformative tools

a) Gene Discovery

Develop tools for monitoring gene expression (qPCR) of immune molecules.

- anti-microbial peptides (AMPs)

b) Tissue Stimulation

Develop protocols for inducing and monitoring immune gene expression.

- Poly I:C, Lipopolysaccharide, β- glucan

2) Ontogeny Study

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

3) Vaccination with Challenge

Monitor the immune response to potential pathogens such as *Vibrio anguillarum.*

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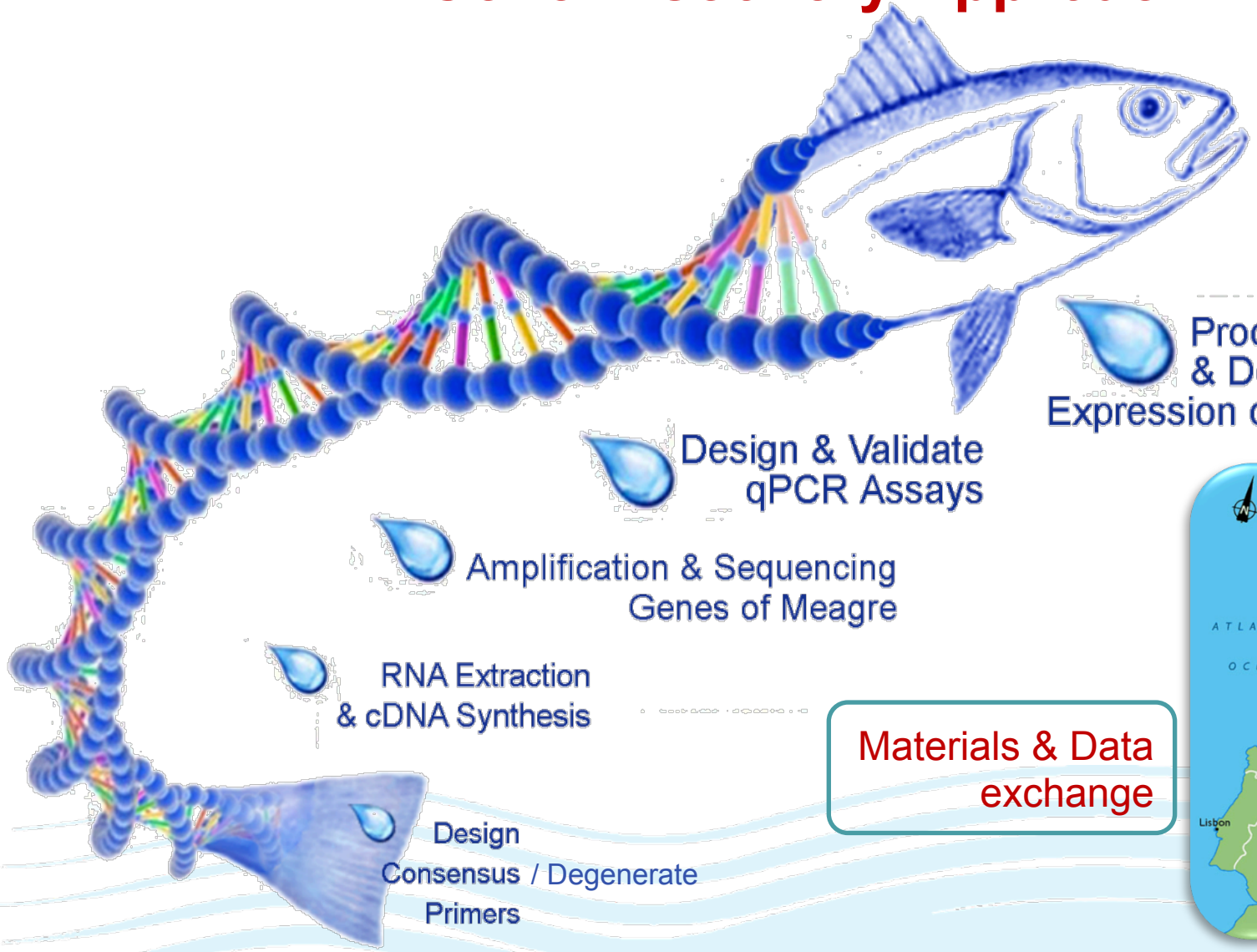
Gene Discovery Approach



Process Fish Tissues & Determine Relative Expression of Immune Genes

Design & Validate qPCR Assays

Amplification & Sequencing Genes of Meagre

RNA Extraction & cDNA Synthesis

Design Consensus / Degenerate Primers

Materials & Data exchange

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Sampling Schedule

Larval and post-larval stage ☐:
Twice weekly sampling during the first 60 days.

Each sample:

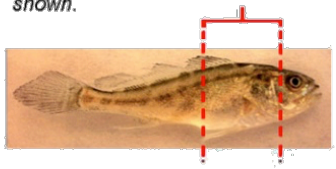
30 larvae collected in RNA later
5 larvae collected in formol
(Total n = 16)

Weaned Juvenile stage ☐:
Weekly sampling after weaning.

Each sample:

10-20 fish * spleen, head kidney, peripheral blood in RNA later
5 fish collected in formol
(n = 8x2 = 16)

** Until fish are large enough for organ dissection collect all organ tissues as a unit as shown.*

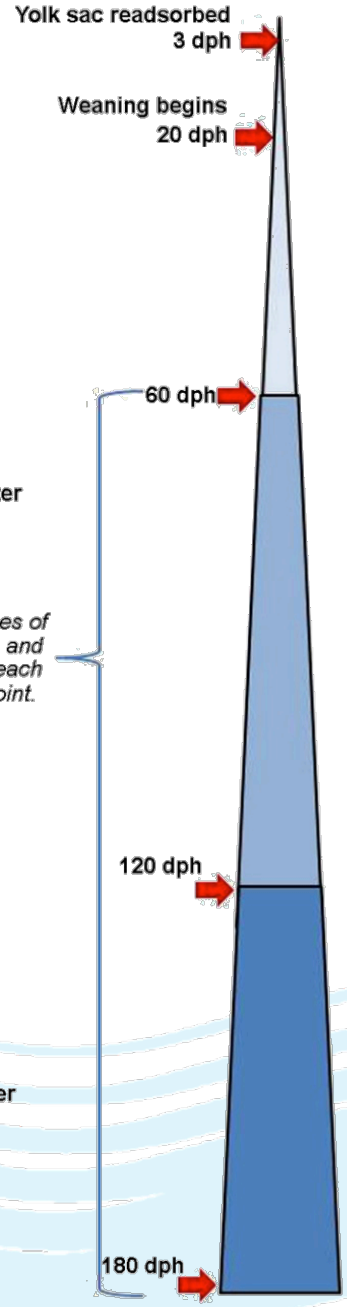


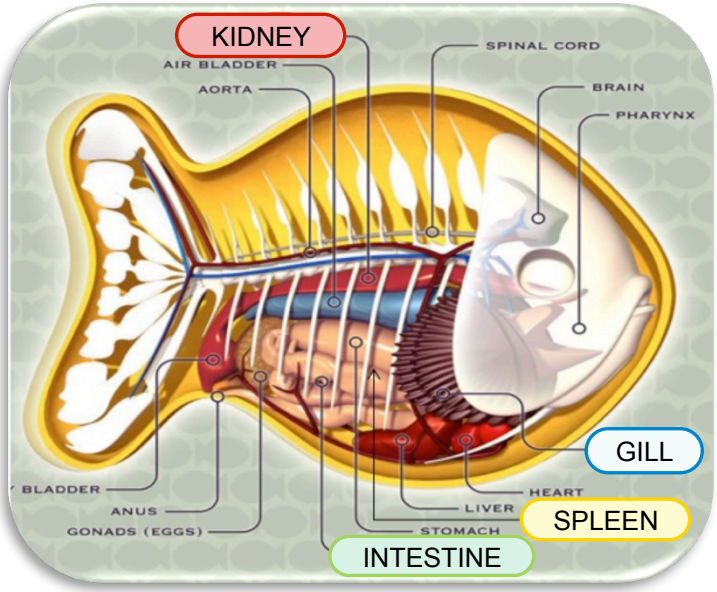
Collect samples of medium size and large size at each sampling point.

Mature Juveniles ☐:
Continue sampling every two weeks until 180 dph.

Each sample:

10-20 fish--spleen, head kidney, peripheral blood, in RNAlater
5 fish collected in formol
(n = 4x2 = 8)





80 – 180 days: tissues from individual dissected organs.

- Kidney
- Gills
- Spleen
- Intestine

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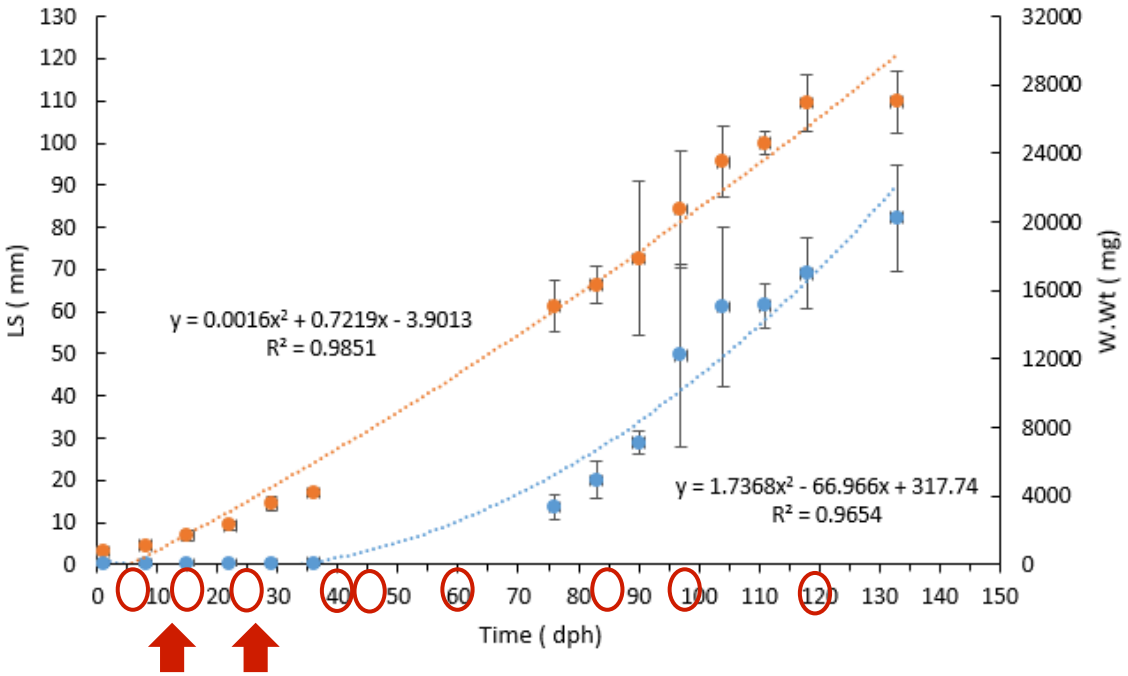
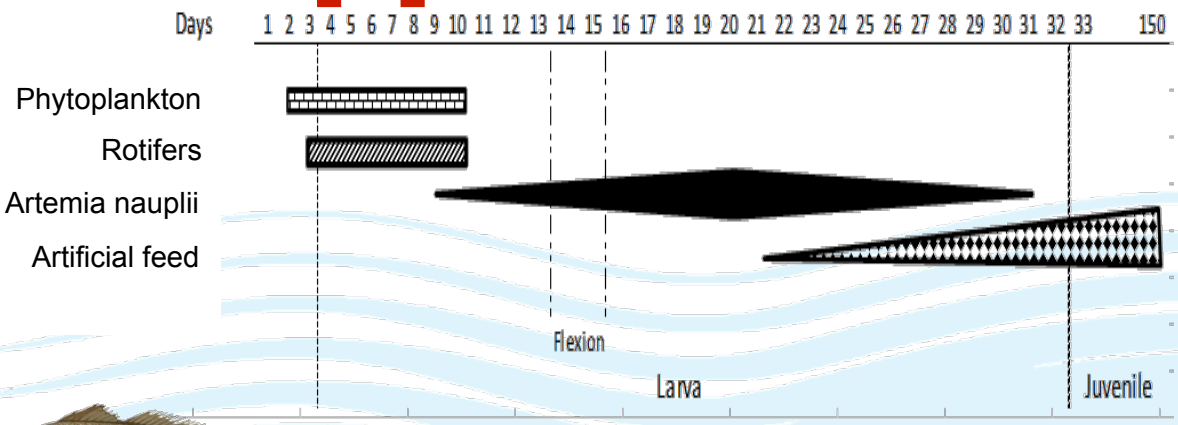
Larval Growout

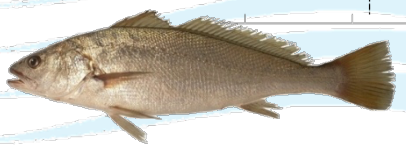


= standard length

= wet weight

= analyzed samples





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Immune Gene qPCR Panel

	Immune function	Amplicon Size (bp)	Efficiency % E
Endogenous Controls	18S ribosomal RNA	140	100.10
	elongation factor 1a (EF1a)	186	100.80
	glyceraldehyde phosphate dehydrogenase (GAPDH)	109	100.00
	Beta Actin	212	100.05
	Hypoxanthine-guanine phosphoribosyltransferase (HPF	160	100.95
Target Genes	Metallothionein	70	102.20
	Lysozyme	148	100.0
	Hepcidin	140	95.90
	piscicidin	111	101.95
	defensin	138	102.30
	C3 complement (C3)	120	100.10
	MX protein (MXP)	187	98.20
	nucleotide oligomerizing domain 2 (NOD 2)	~	~
	nucleotide oligomerizing domain 3 (NOD 3)	154	100.80
	tumor necrosis factor 1a (TNF1a)	104	99.20
	Immunoglobulin M (IgM)	304	99.85
	Immunoglobulin T (IgT)	104	100.00
	recombination activating gene (RAG1)	144	100.40
	Myeloid differentiation primary response 88 (MyD 88)	120	102.20
	T-cell receptor beta, heavy chain (TCRb)	281	103.15
	cyclooxygenase (COX2)	211	100.50
	Interleukin 1B (IL-1b)	103	99.60
	Interleukin 4/13 (IL-4/13)	107	99.00
	Interleukin 10 (IL-10)	187	102.15
	Interleukin 17 (IL-17)	91	96.10
	Interleukin 22 (IL-22)	146	101.75
	Interferon type 1 (INF 1)	178	100.10
	Interferon gamma (INF g)	171	99.85

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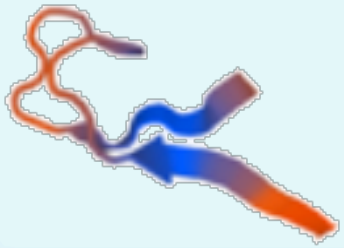
Innate Defenses - antibacterial

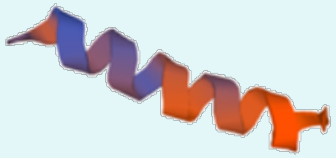
antimicrobial peptides (AMPs) & lysozyme

β- Defencin
42 aa

Hepcidin
26 aa



Piscidin
25 aa



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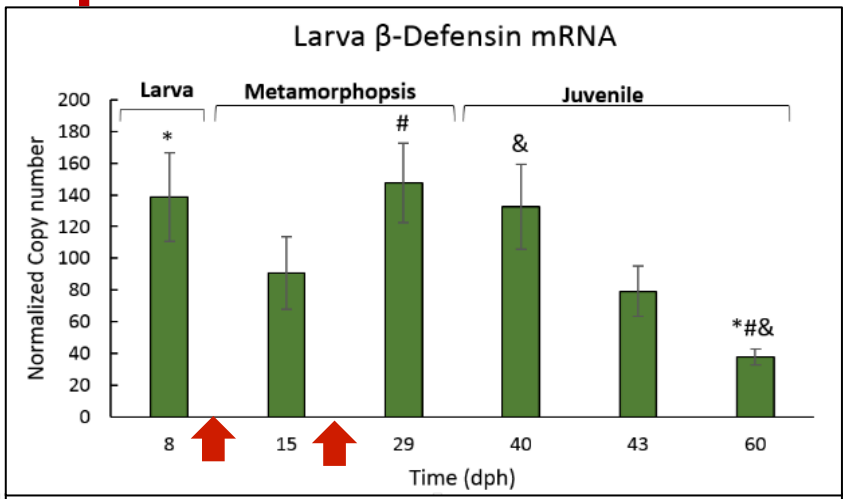
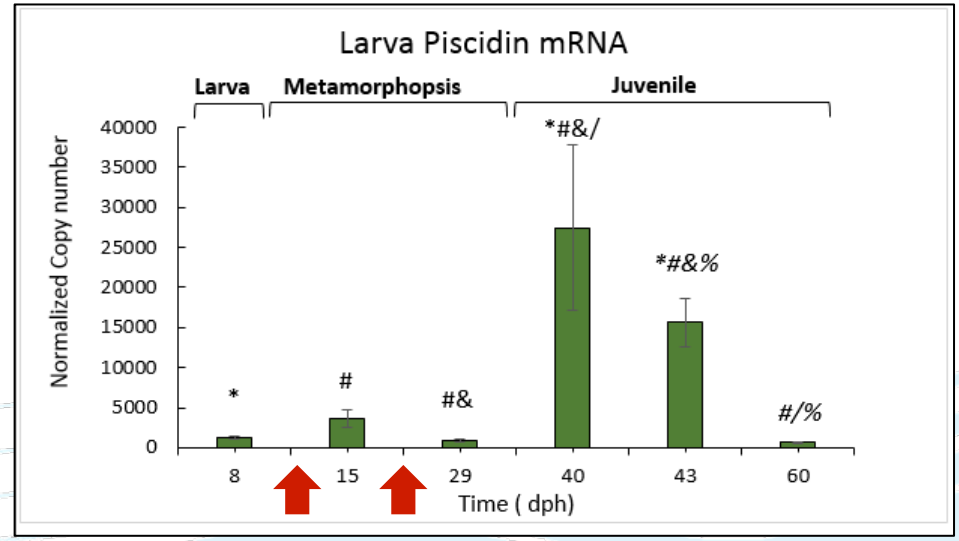
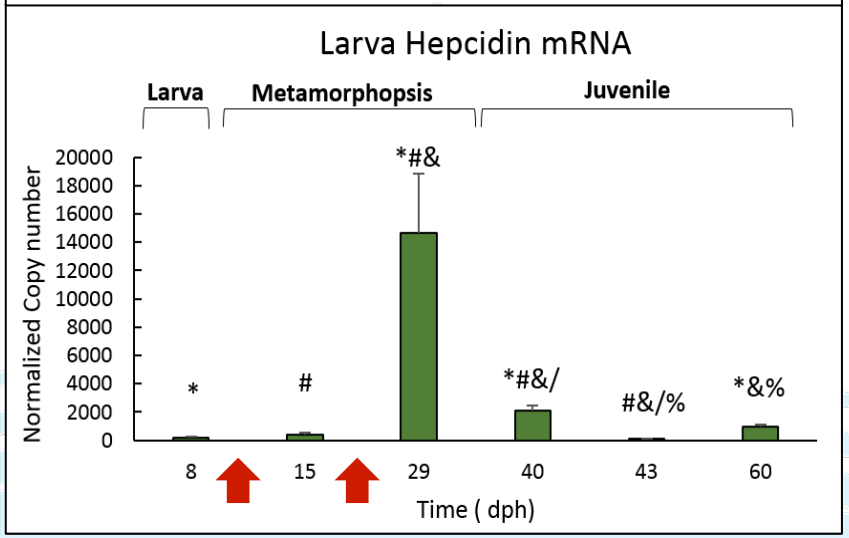
Ontogeny of Antimicrobial Protein Gene Expression

Characterization of AMP expression:

- ➢ Analysis of whole animals up to ~4 g w.wt.
- Changes in diet (exposure to microbial antígens) seem to correlate with changes in gene expression







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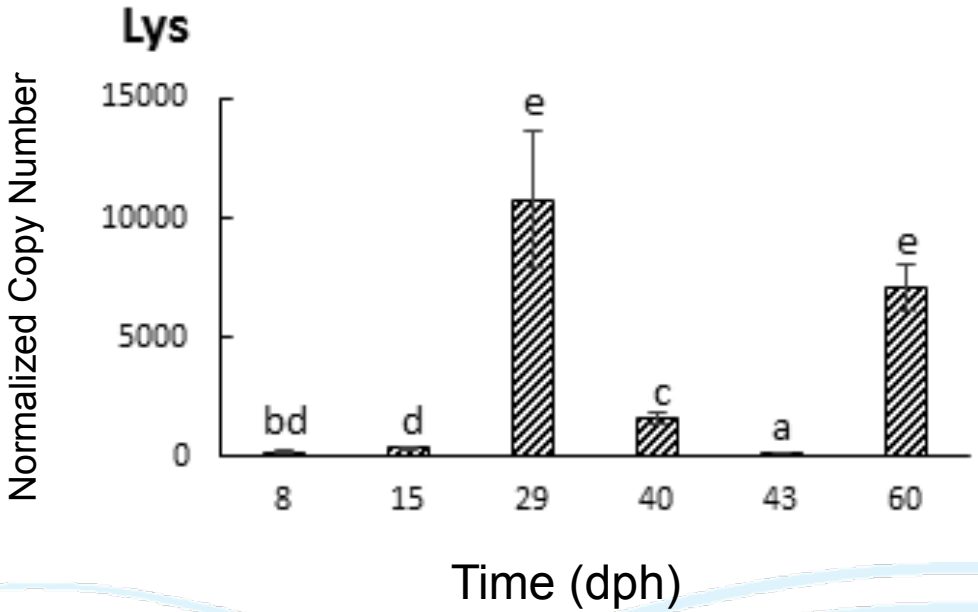
Innate Defenses – antibacterial lysozyme

Characterization of LYS expression:

- Large spike in expression after weaning begins



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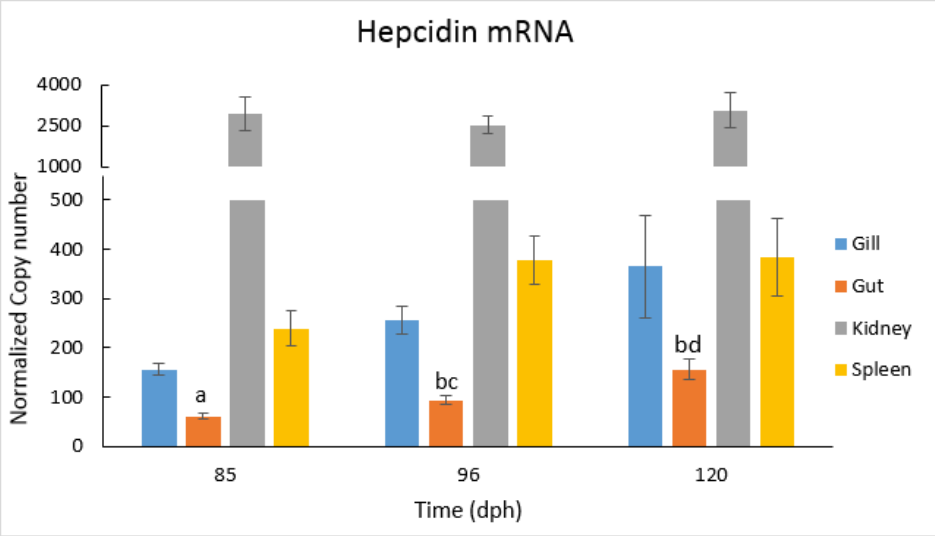
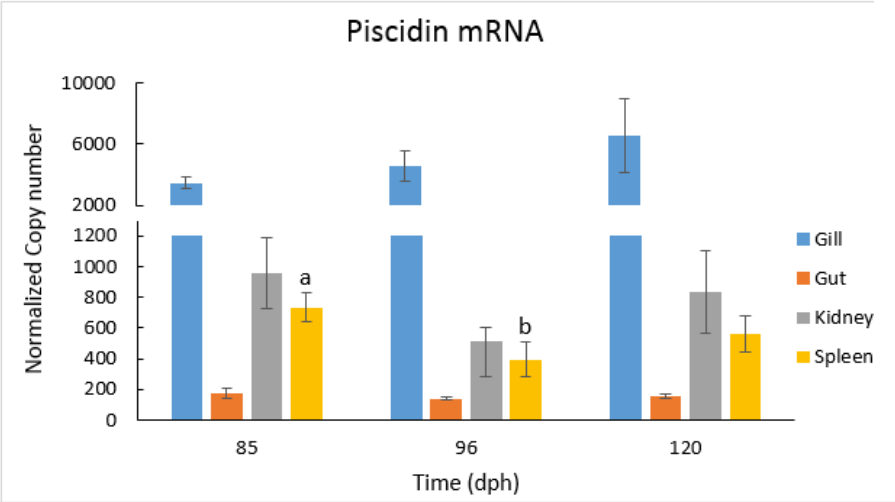
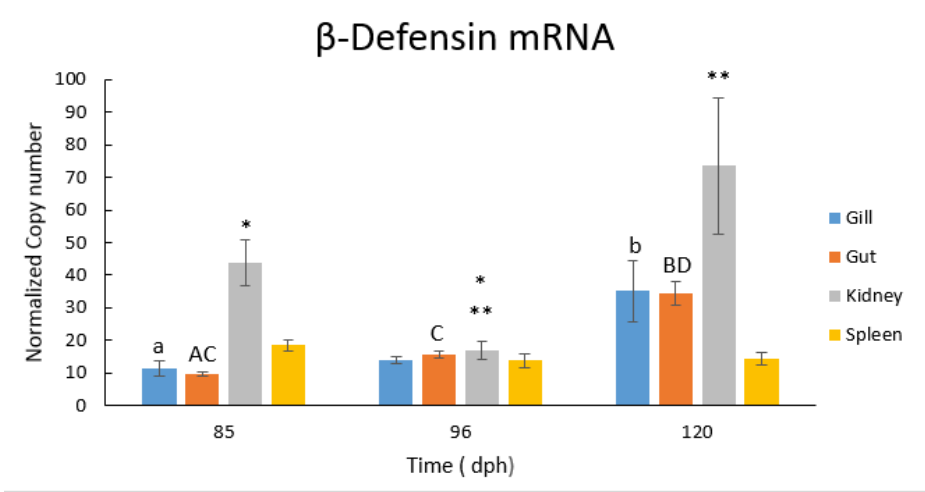
Ontogeny of AMP Genes

Characterization of AMP expression:

- ➢ Dissected organs of juvenile – mature
- • Expression of AMPs in the gut appear to be under tight control, possibly as a mechanism to avoid harm to the beneficial bacteria in the gut microbiota
- • Hepcidin highest expression in kidney
- • Piscidin highest expression in gills







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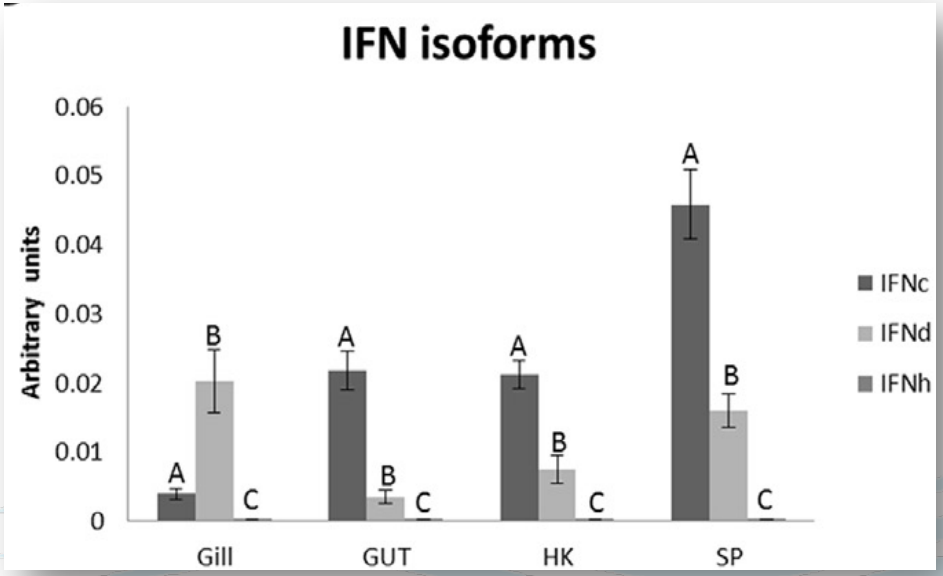
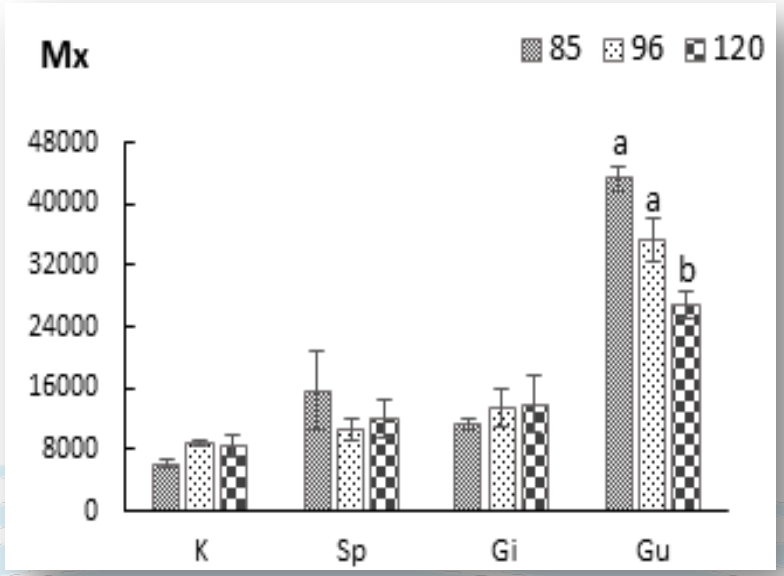
Innate Defenses – antiviral

interferon (IFN) & MX protein genes

Characterization of multiple IFN genes identified:

- Constitutive expression of IFNs are regulated distinctly in each tissue examined
- IFNc expression in the gut correlates with constitutive expression of MXP in the gut.
- IFNh is expressed at very low levels.





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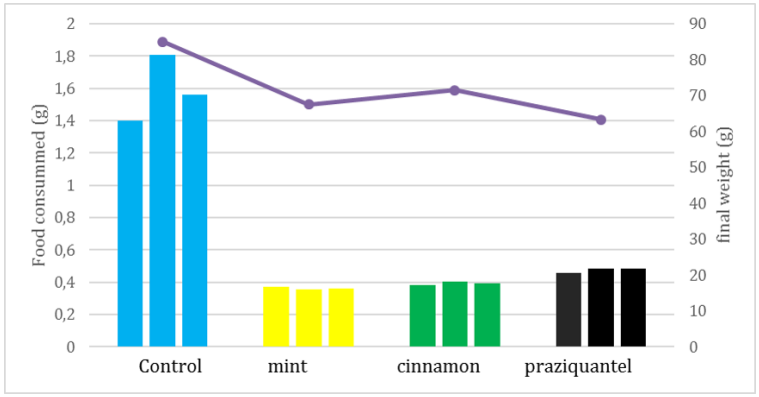
Parasiticide Treatments

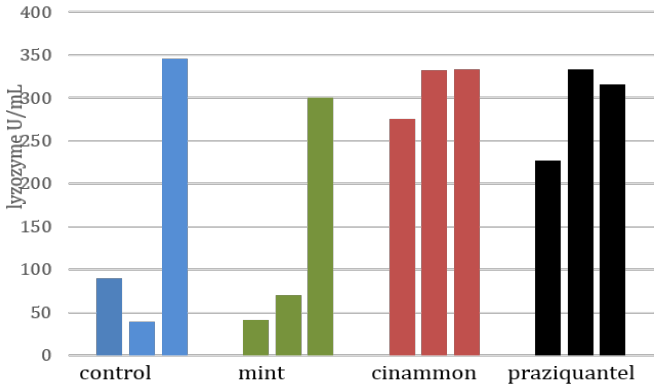
Essential Oils with vermicide activity used in this study:

3 replicate tanks/ treatment; 10 fish/ tank

- Control Diet without essential oil (EO)
- Diet + Mint EO
- Diet + Cinnamon EO
- Diet + Prazinquantel – as a control for positive parasiticide activity



Final mean weight per treatment (line). Average food consumption per tank over a 6-week period (bars).



Levels of lysozyme measured per treatment.

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Parasiticide Treatments

Cinnamon EO compared for immune stimulation activity:

Trematode eggs seeded into tanks to infect experimental fish (n= 15/tank)

- Control diet without EO
- + Cinnamon EO
- + Echinacea – as a control for immuno-stimulatory activity

Treatment	Prevalence (%)	Intensity (>5 ind/ gill)	Intensity (>8 ind/gill)
Control	100	15	2
Echinacea	100	15	10
Cinnamon	60	0	0

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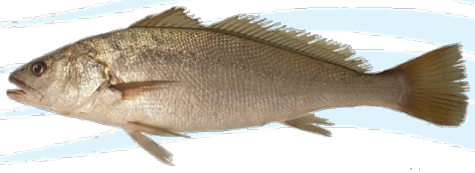
Summary

- **Immune system**: in terms of immune gene functions fish become functionally competent at 86 dph. Weaning stimulates significant alterations in expression of some immune-related genes.
- **Antimicrobial peptide genes** with constitutive expression in tissues examined.
  - High expression of piscidin in gills; broad-spectrum activity against bacteria & ciliated protozoans.
- **Interferon**: three isoforms identified that are expressed in a tissue-specific manner. Possibly represents a more efficient anti-virus response – needs study.
- **Cinnamon EO** appears to provide some positive benefits for prevention of parasite infestations (trematodes) with minimum negative side effects for digestive physiology.



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