MICROBIOLOGY AND IMMUNOLOGY OF LARVICULTURE

Peter Bossier, O. Vadstein, Y. Olsen, N. Boon, O. Bergh, F. J. Gatesoupe, S. Picchietti, L. Abelli, E. Buonocore, A.M. Fausto, G. Scapigliati, J. Galindo-Villegas, V. Mulero, P. Makridis, P. Sorgeloos, T. Defoirdt, K. Dierckens

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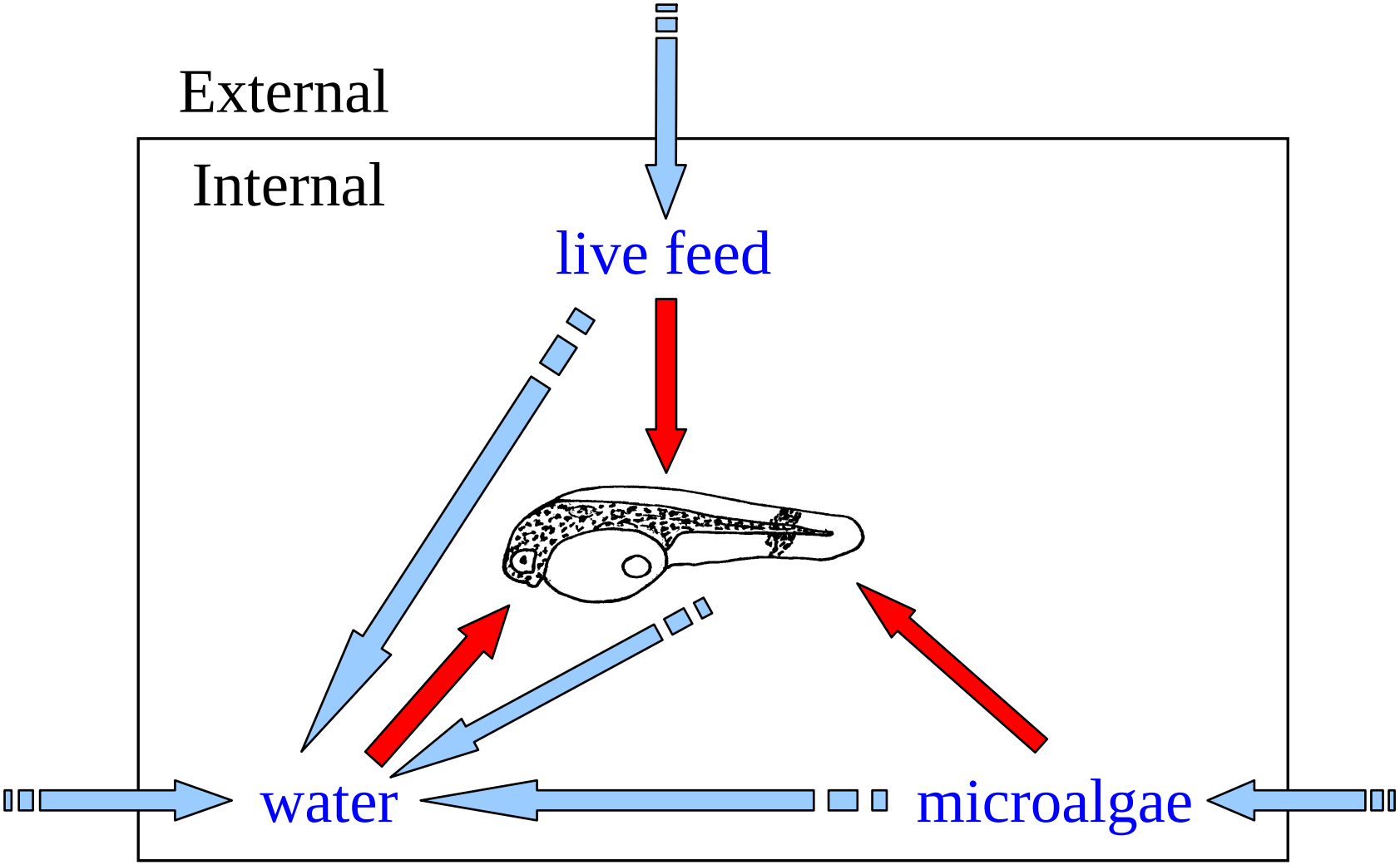
supported by LARVANET

Overview of the presentation

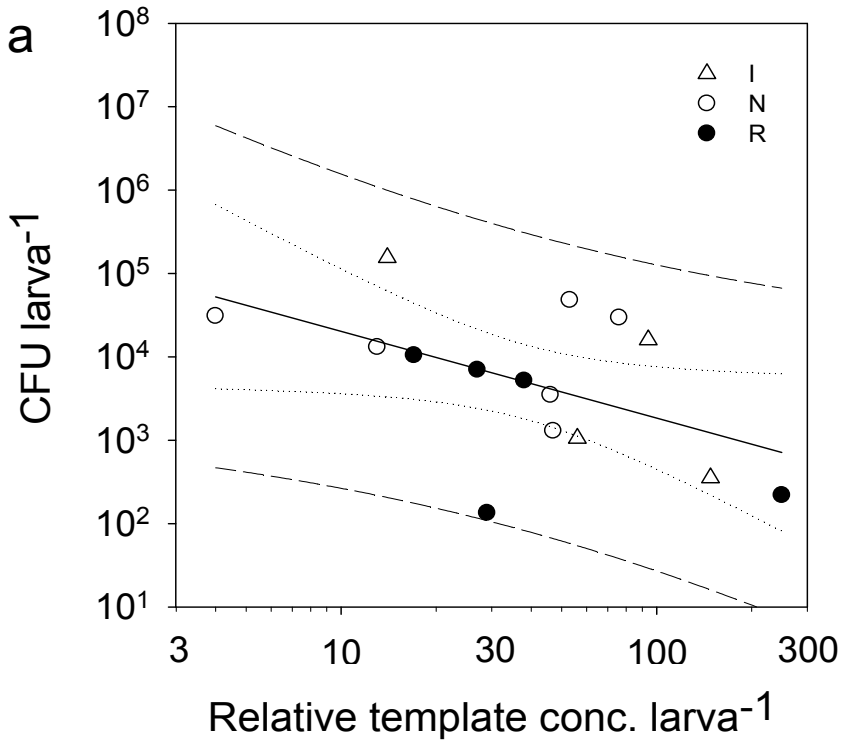
- The microbial ecosystem of larvae
- Methodological aspects of characterisation of microbial community (MiC)
- Detrimental host/microbe interactions
- Immunology, immunological ontogeny and immunological modulation in larvae
- Steering larval microbial communities to the benefit of the host

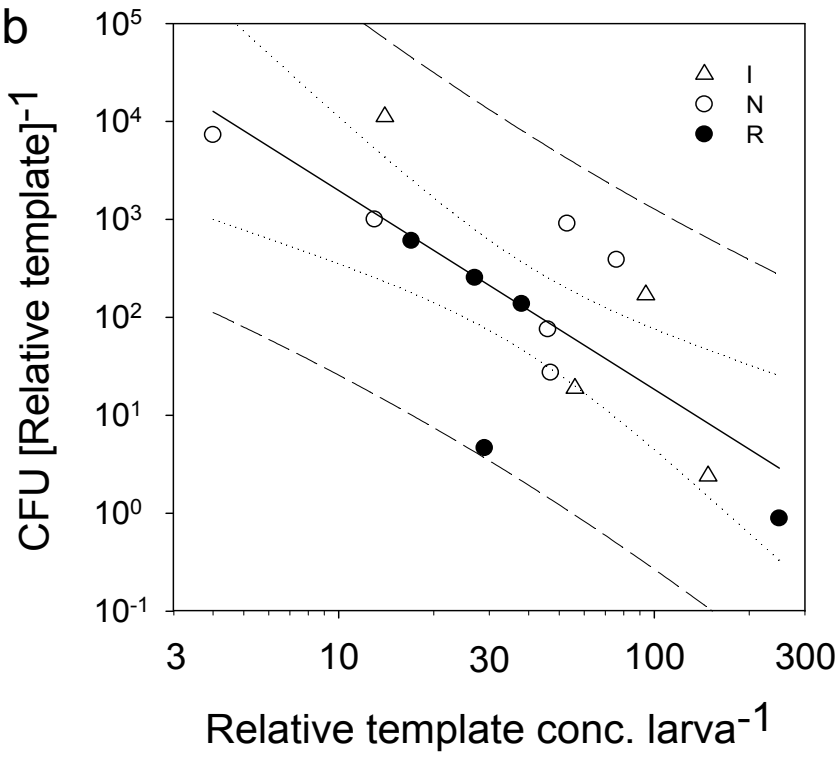
Main point: Microbial communities develop in an ”ecosystem”



Vadstein *et al*. (2004).

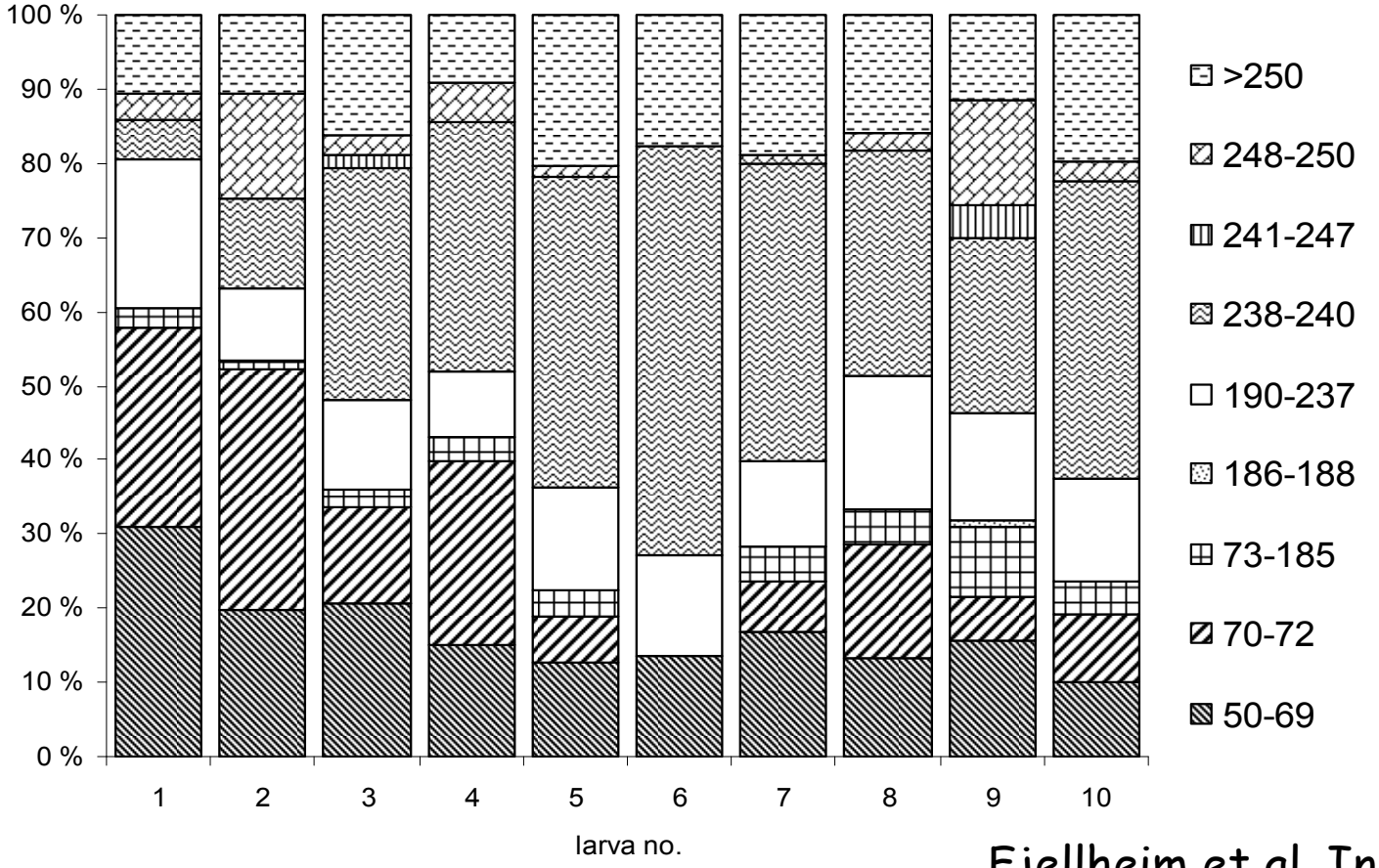
Culture dependent vs. independent





Fjellheim et al. In prep

Individual variability of bacterial community: Cod larvae with T-RFLP



Fjellheim et al. In prep

The microbial ecosystem of larviculture

- High load of micro-organisms
- Probably large fluctuations in composition and numbers within and between individuals (even in the same tank)
- Strong stochastic factor
- Need to analyse MiC at the individual level
- Experimental approach: gnotobiotic system

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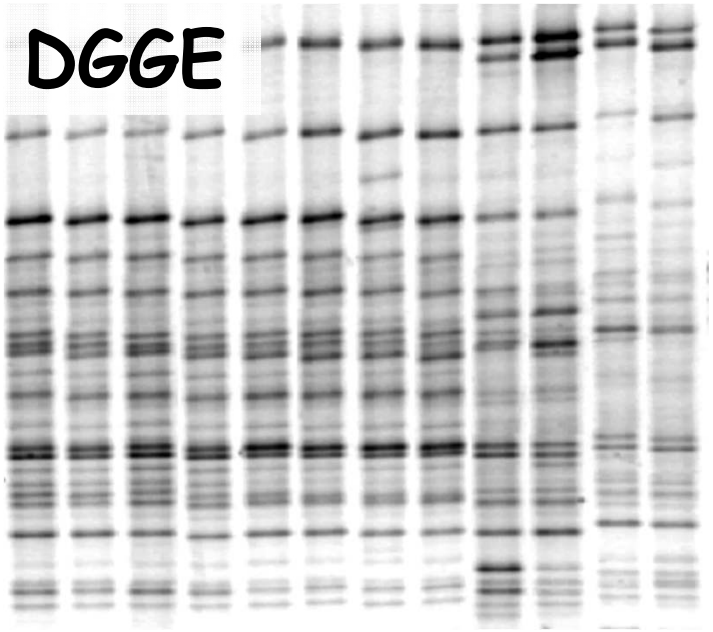
Microbial Resource Analysis

Provide a new tool of conceptual interpretation of the 16S rRNA molecular fingerprinting pattern, based on a pragmatic processing through three levels of analysis:

- **Range-weighted richness (Rr)**
- **Dynamics (Dy)**
  - ➔ Moving Window Analysis
  - ➔ Rate of Change: $\Delta_{t(week)}$
- **Functional Organization (Fo)**
  - ➔ Lorenz curves
  - ➔ Gini coefficient

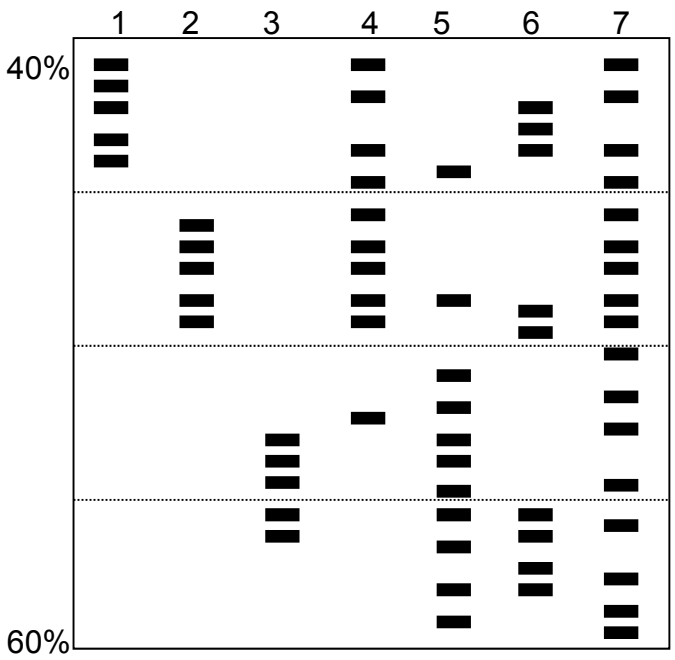


Marzorati, M., et al (2008). *Environ Microbiol* **10**, 1571-1581

Concept 1: Range-weighted richness Rr

$$Rr = N^2 \times D_g$$

N represents the total number of bands in the pattern
D_g the denaturant gradient comprised between the first and the last band of the pattern.

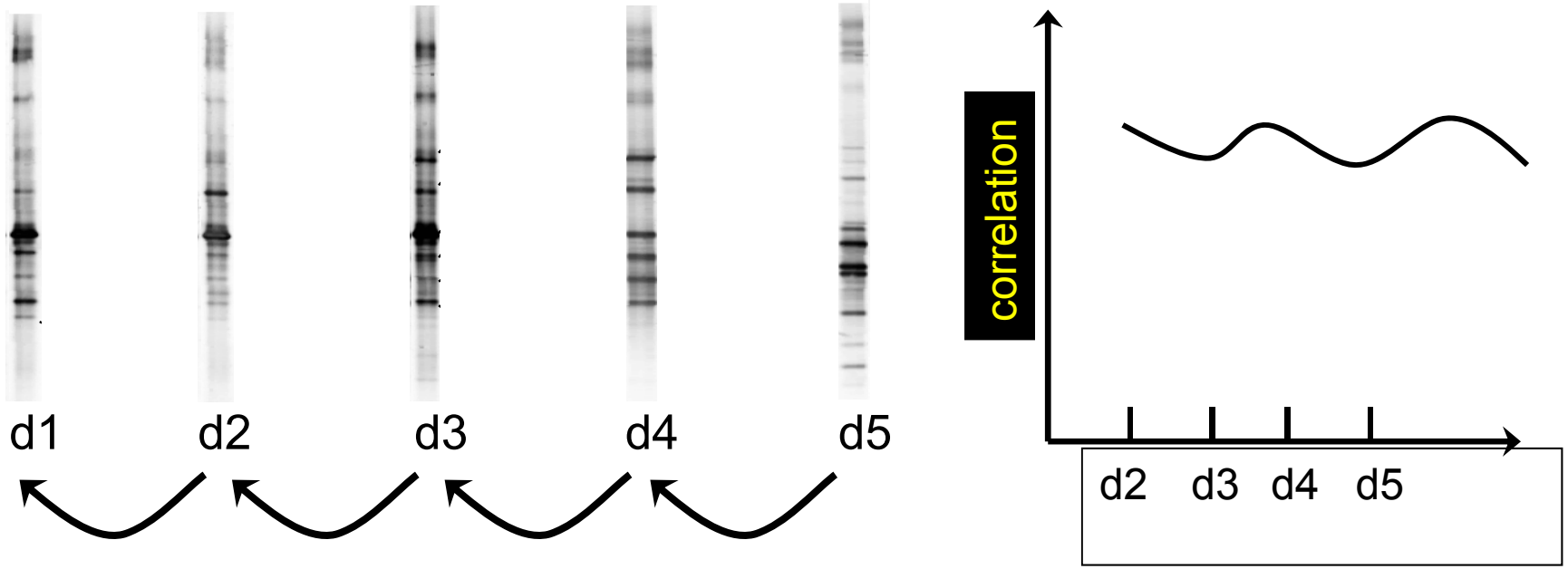


Environment	Denaturing gradient	Range No. bands	*Rr* [a]
Insect (*Scaphoideus titanus*)	40–60%	3–5	0.3 ± 0.4
Vagina	30–50%	2–10	2.2 ± 2.9
NR-SOB in CSTR[b]	45–60%	4–9	2.6 ± 1.4
Subgengival plaque	20–80%	2–6	4.2 ± 3.2
Deep-sea hydrothermal site	35–38%	11–14	4.6 ± 1.2
1,2-DCA-contaminated groundwater	30–60%	3–10	4.9 ± 4.4
Insect gut (*Vespula germanica*)	40–75%	4–7	5.6 ± 2.4
Oil-contaminated soil[c]	35–80%	6–8	8.1 ± 3.2
Sulfidogenic anaerobic bioreactor[d]	20–80%	7–10	23 ± 11
Pharmaceutical activated sludge	45–60%	12–17	25 ± 11
Artic sea ice	20–70%	10–11	26 ± 3
Puffer fish ovary	30–60%	11–15	29 ± 14
Coastal seawater	40–80%	8–20	31 ± 23
Human colon descendens	45–60%	13–17	39 ± 9
Compost-packed benzene biofilter[e]	40–60%	9–18	40 ± 16
Soil crust of sand dunes	40–60%	10–13	42 ± 17
Gut of Chinese mitten crab	25–55%	13–16	50 ± 17
Municipal activated sludge	50–65%	18–19	57 ± 11
Legumes rizosphere	54–64%	22–34	78 ± 30
Intertidal sediments	40–50%	27–31	129 ± 18
Stable nitrifying reactor	45–60%	24–41	145 ± 59
Garden soil	40–70%	23–32	220 ± 63

Marzorati, M., et al (2008). *Environ Microbiol* **10**, 1571-1581

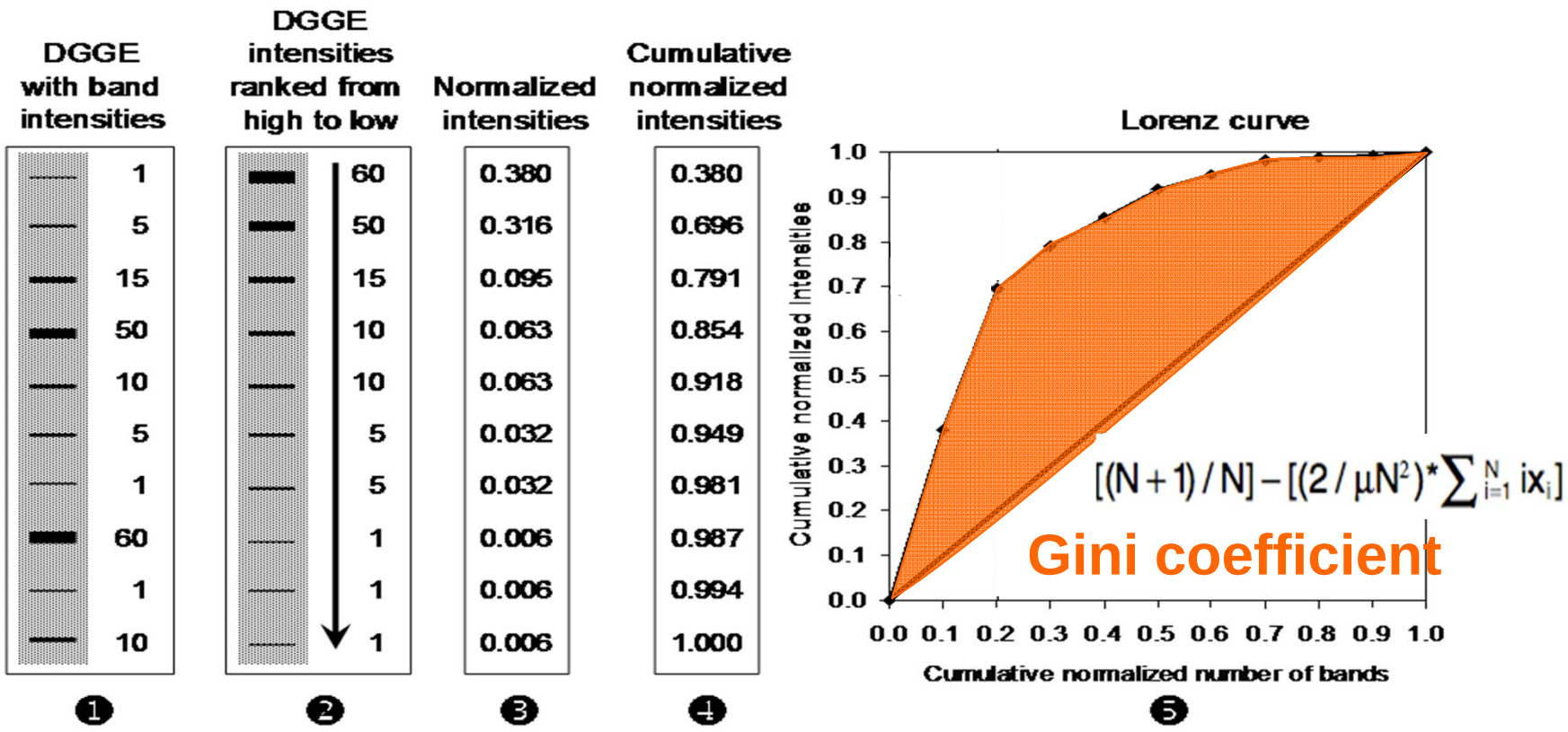
Concept 2: Quantifying Dynamics Dy

❖ **M**oving **W**indow **A**nalysis (Wittebolle et al., JAM, 2005)



Concept 3: Functional Organisation Fo

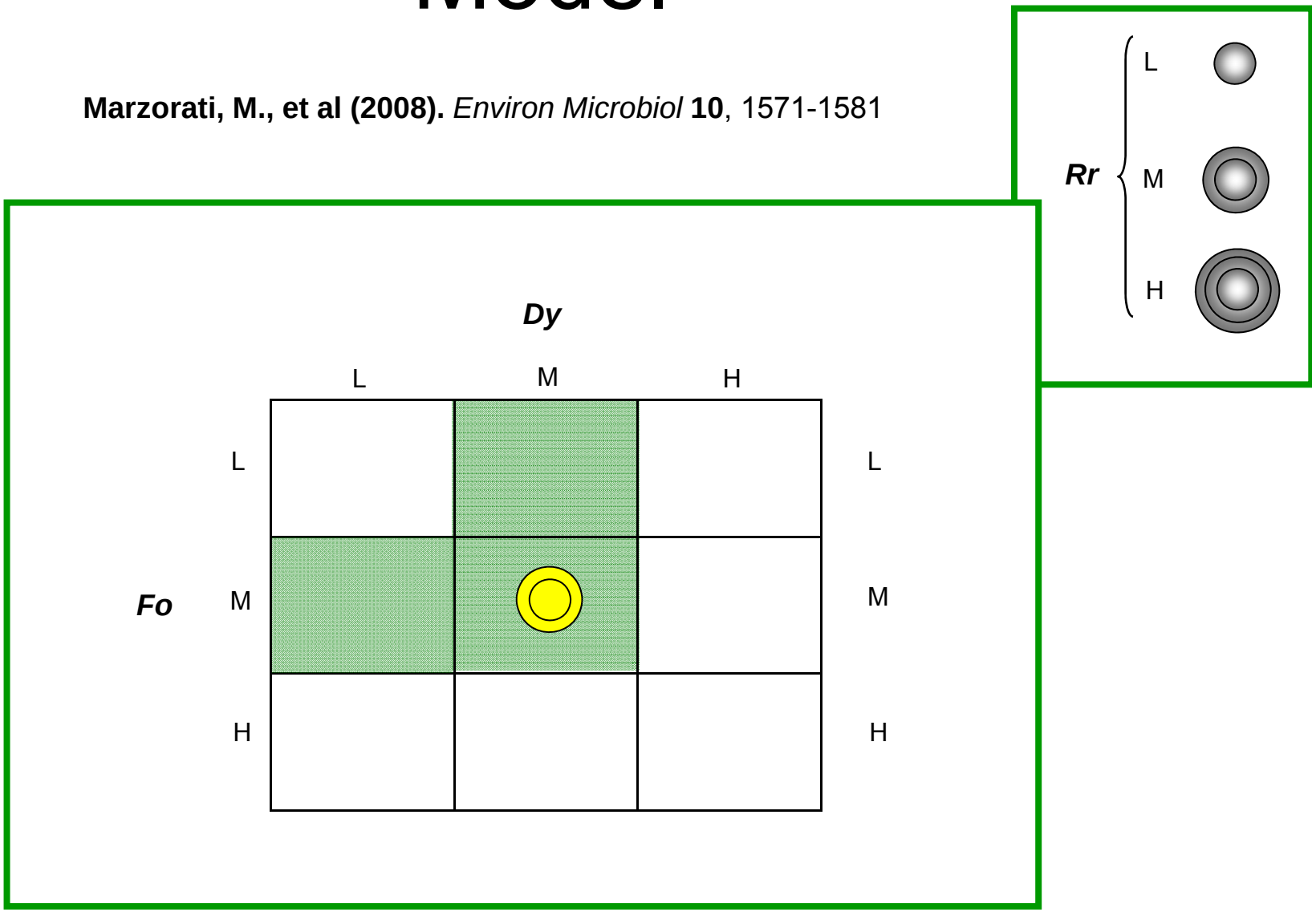
Lorenz curves to describe the evenness



Mertens, et al. (2005). *Environ Microbiol* **7**, 660-669.

Model

Marzorati, M., et al (2008). *Environ Microbiol* **10**, 1571-1581



Conclusions

- Microbial community evolves constantly f(time)

  ➔ 1 analysis = capture of that moment !

- Microbial diversity: pragmatic processing
  - Richness
  - Dynamics: MWA & $\Delta_{t(week)}$
  - Evenness/Internal structure: Lorenz & Gini
  - **<u>Can be linked to community functioning</u>**

<u>Perspectives</u>

➔ **Define 'health' limits for each biotope/reactor/organism**

➔ **These 'health' limits are not determined in aquaculture**

Problem

- PCR based techniques (DGGE) tends to detect the presence of micro-organisms above the 1%
- Hence minor components of the microbial community are ignored.
- Can we state that they do not have an influence on the host?
- Processing DGGE data in time can not be done at the individual level for larvae (destructive sampling)

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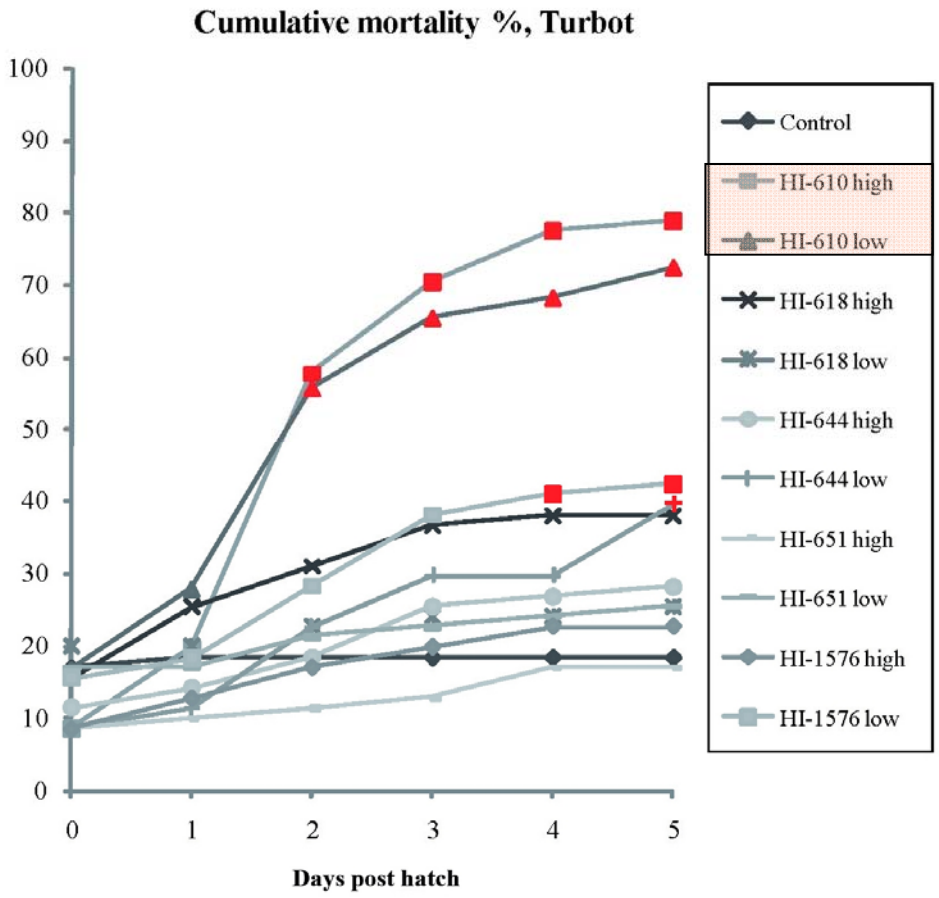
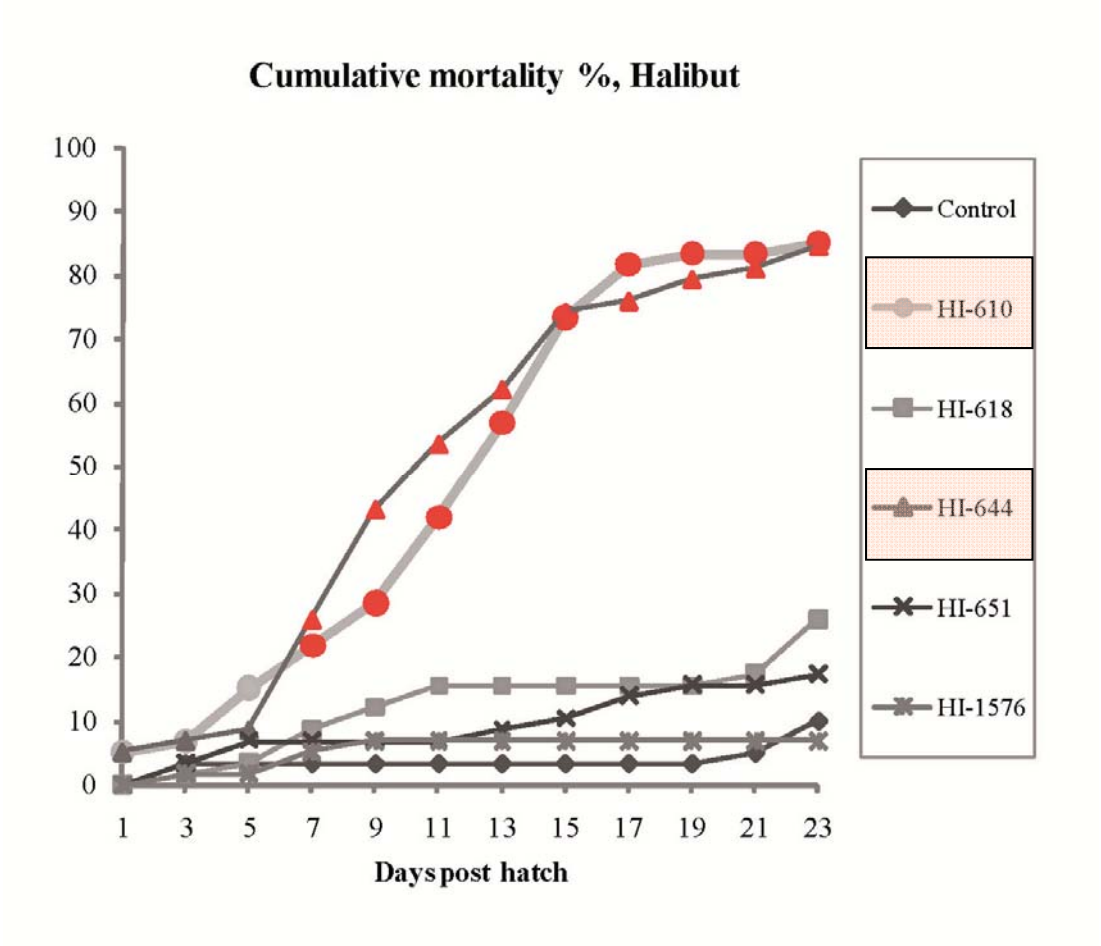
Turbot – V. anguillarum



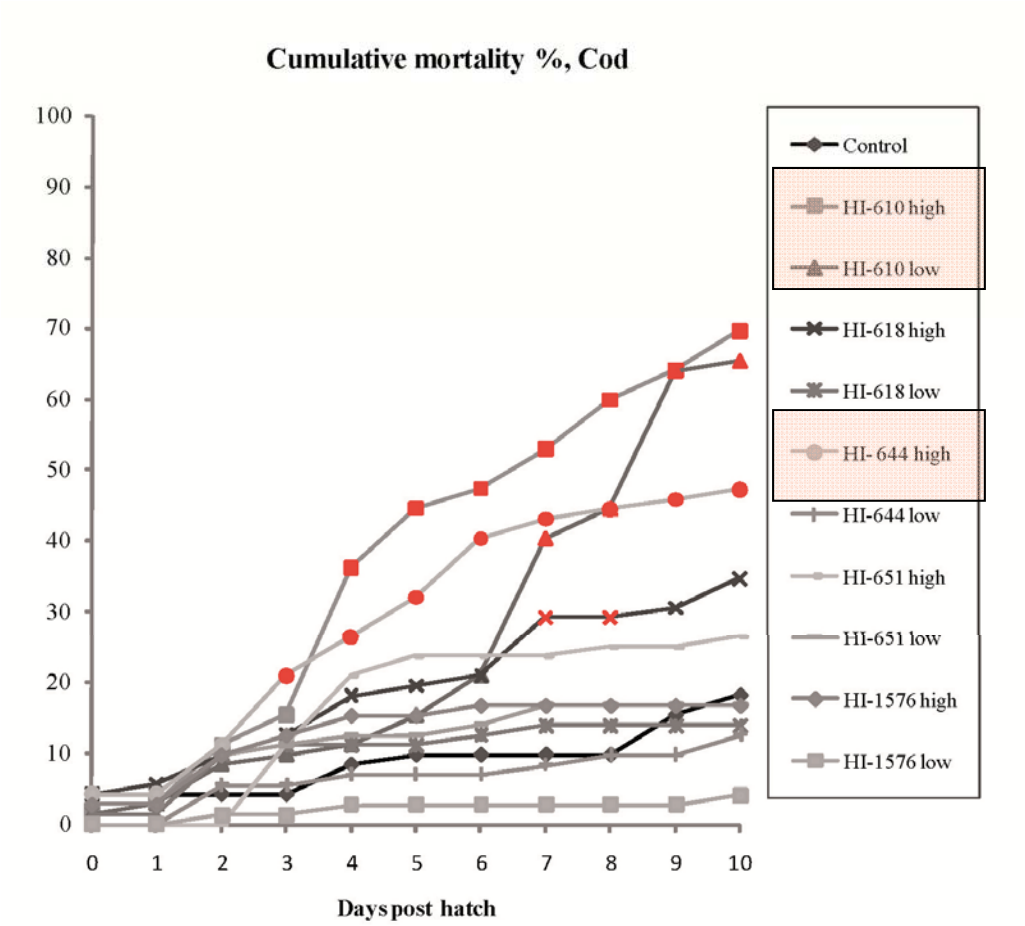
Strain	serotype
HI-610	O2α
HI-618	O2β
HI-644	O1
HI-651	V. s
HI-1576	V. sp

Halibut – V. anguillarum



Strain	serotype
HI-610	O2α
HI-618	O2β
HI-644	O1
HI-651	V. s
HI-1576	V. sp

Cod- V. anguillarum



Strain	serotype
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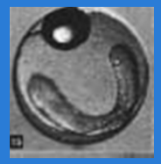
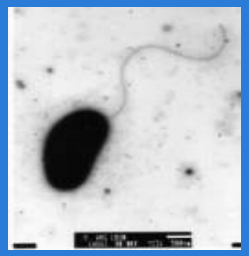
Gnotobiotic Artemia –seabass food chain



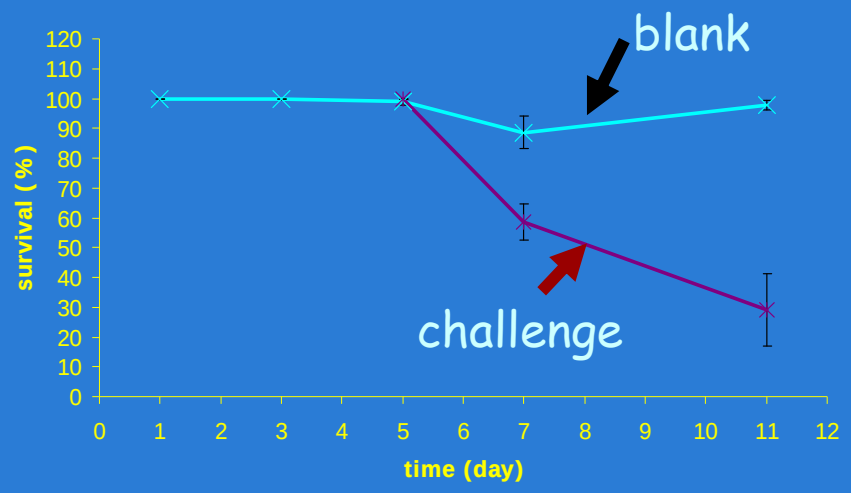


Vibrio anguillarum

At 16°C

Dierckens et al, 2009

Gnotobiotic Artemia –seabass food chain



Virulent

Vibrio anguillarum

Strain HI610

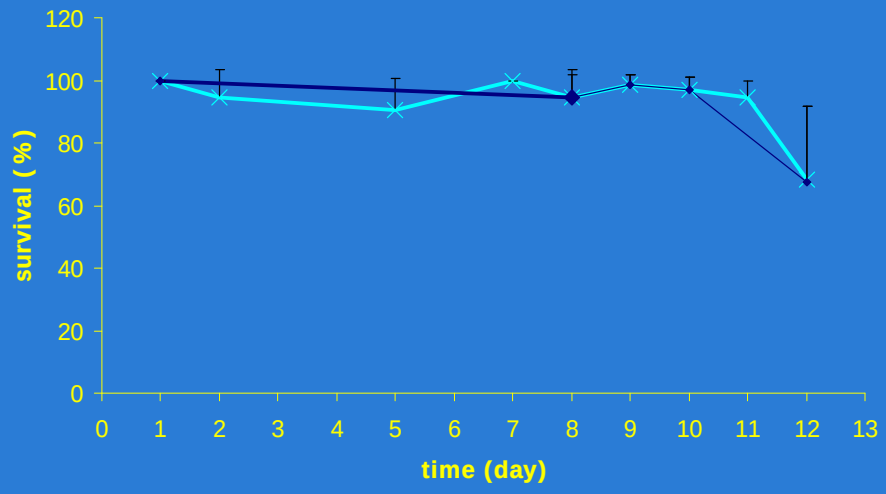
serovar O2a

Avirulent

Vibrio anguillarum

Strain 43

serovar 01



Dierckens et al, 2009

Detrimental host/microbe interactions : conclusions

- Many different serotypes
- Might contain different virulence factors
- Susceptibility is dependent on the host
- Some strain have a broad host range, others a narrow
- Detrimental Host/microbe interaction in each case specific
- *Importance of host “robustness” should not be ignored*

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Disease resistance: Immune system

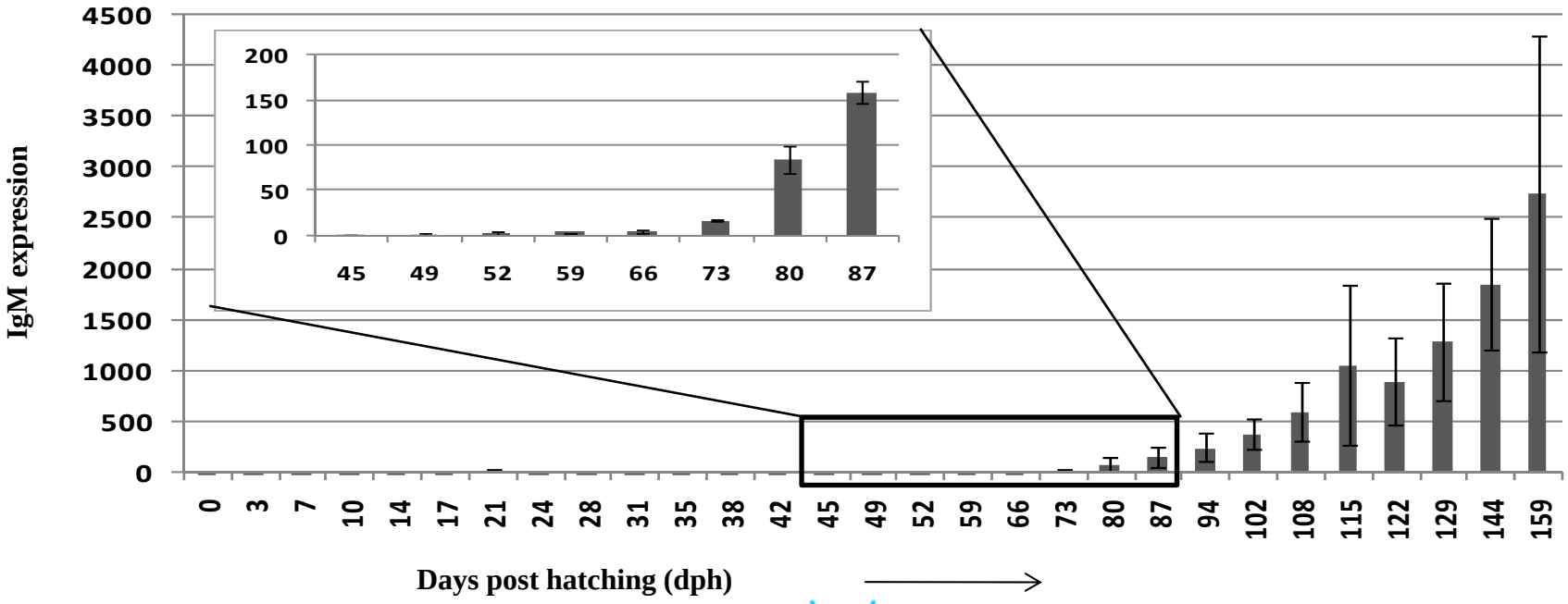
	Non-specific (innate)	Specific (acquired)
Humoral	Lytic enzymes, e.g. lysozyme Complement Agglutinins and precipitins Enzyme inhibitors Growth inhibitors	Antibodies
Cellular	Macrophages/monocytes Granulocytes Non-specific cytotoxic cells	B-cells T-cells

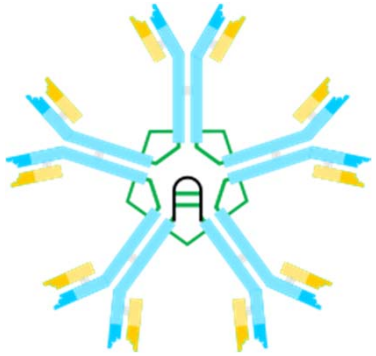
Larvae do not have a specific immunity, except from some of maternal origin

Simplified figure showing the main components of the non-specific and specific immune system.

Ontogeny of adaptive immune system:

IgM type of antibodies in halibut





During ontogenesis, T cells appear simultaneaously in thymus and intestine, remarkably earlier than B cells

Table 2
Immunocytochemical appearance of B and T cells in lymphoid organs of sea bass (in dph, ±SD; at 16 °C) and carp (in dpf at 25 °C; hatching at 2 dpf)

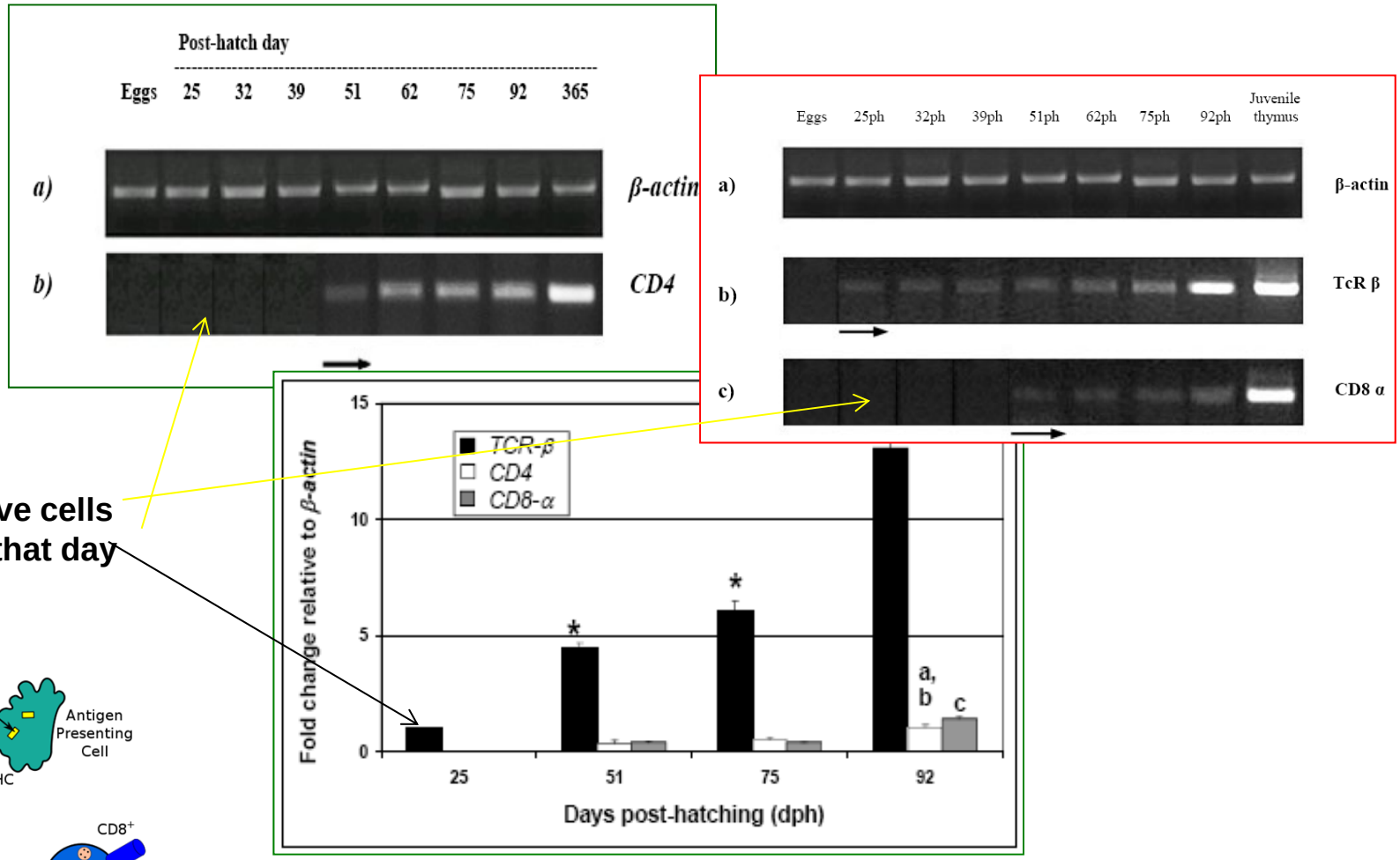
Tissue	T cells (DLT15) sea bass	B cells (DLIg3) sea bass	Ig^{-} lymphoid cells carp	B cells (WCI12) carp
Thymus	28 ± 2	90 ± 5	4[a]	negligible
Head kidney	35 ± 5	45 ± 5	7[a]	14
Spleen	45 ± 3	45 ± 3	7[a]	14
Intestine	28 ± 2	90 ± 5	3[b]	35

The sea bass data are based on ±100 sections/organ, and in carp on immuno-histochemistry and flow cytometry.

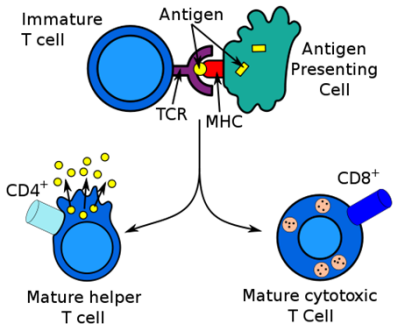
[a] Based on WCL9 (cortical thymocytes in adult carp).

[b] Based on WCL38 (putative mucosal T cells).

lymphocyte differentiation in developing sea bass (18° C)

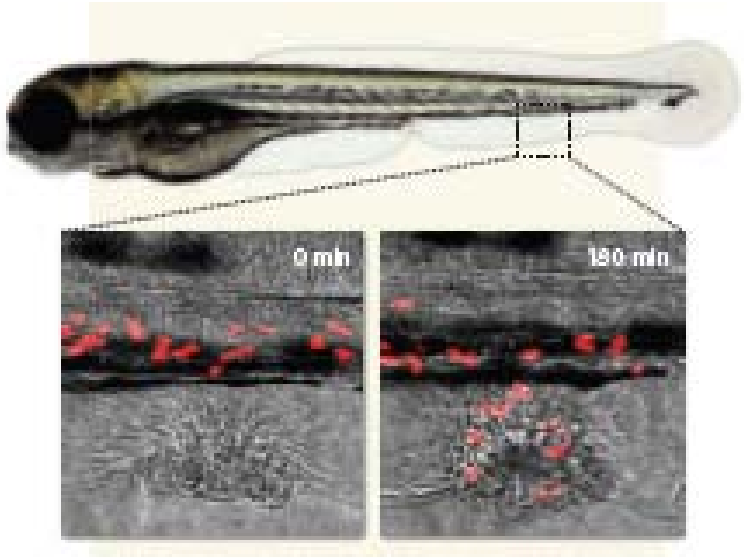


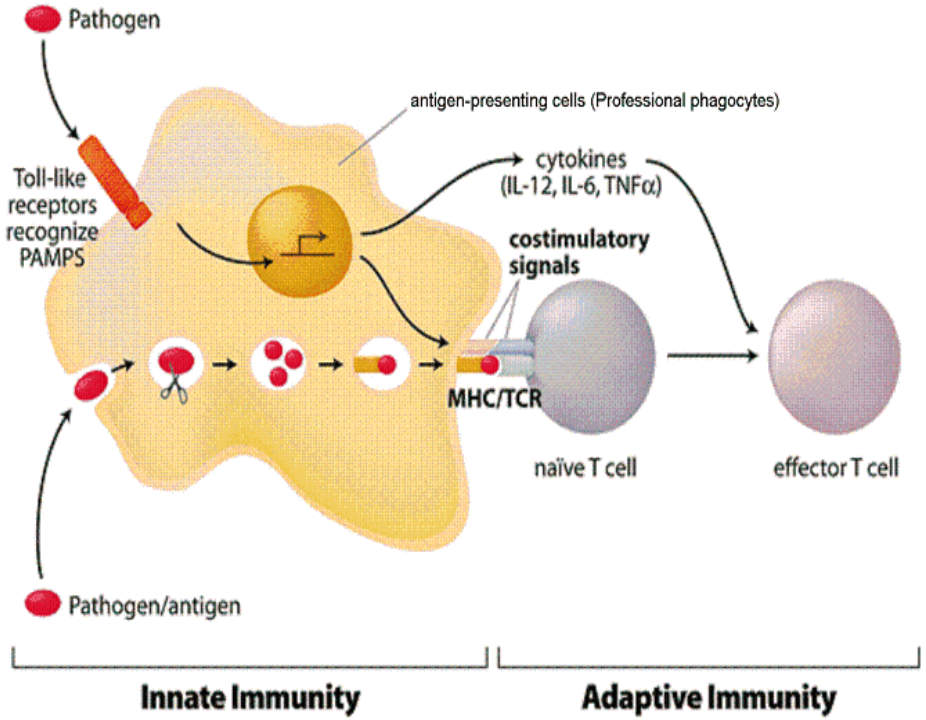
DLT15-posive cells appears at that day



Immunology: professional phagocytes

Most fish rely only on their innate immune capacities during larvae period. However, after 4 to 6 weeks of hatching innate immunity becomes critical to adaptive immune response

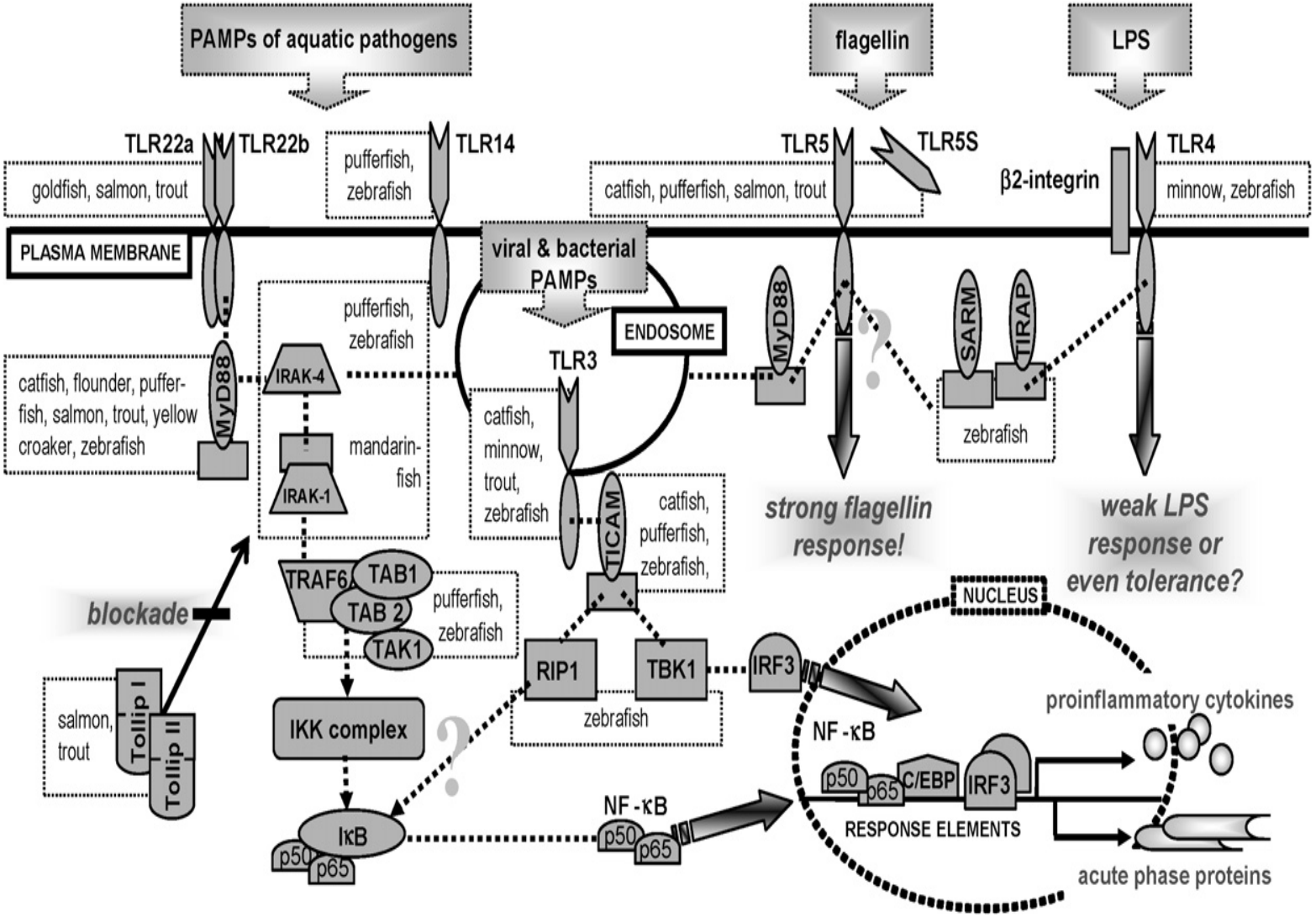




Cellular innate immunity expressed as migration, phagocytosis and release of reactive oxygen species release could be visualized using transgenic zebrafish larvae acidophilic granulocytes expressing a fluorescent reporter

Innate immunity: piscine TLRs signaling cascade

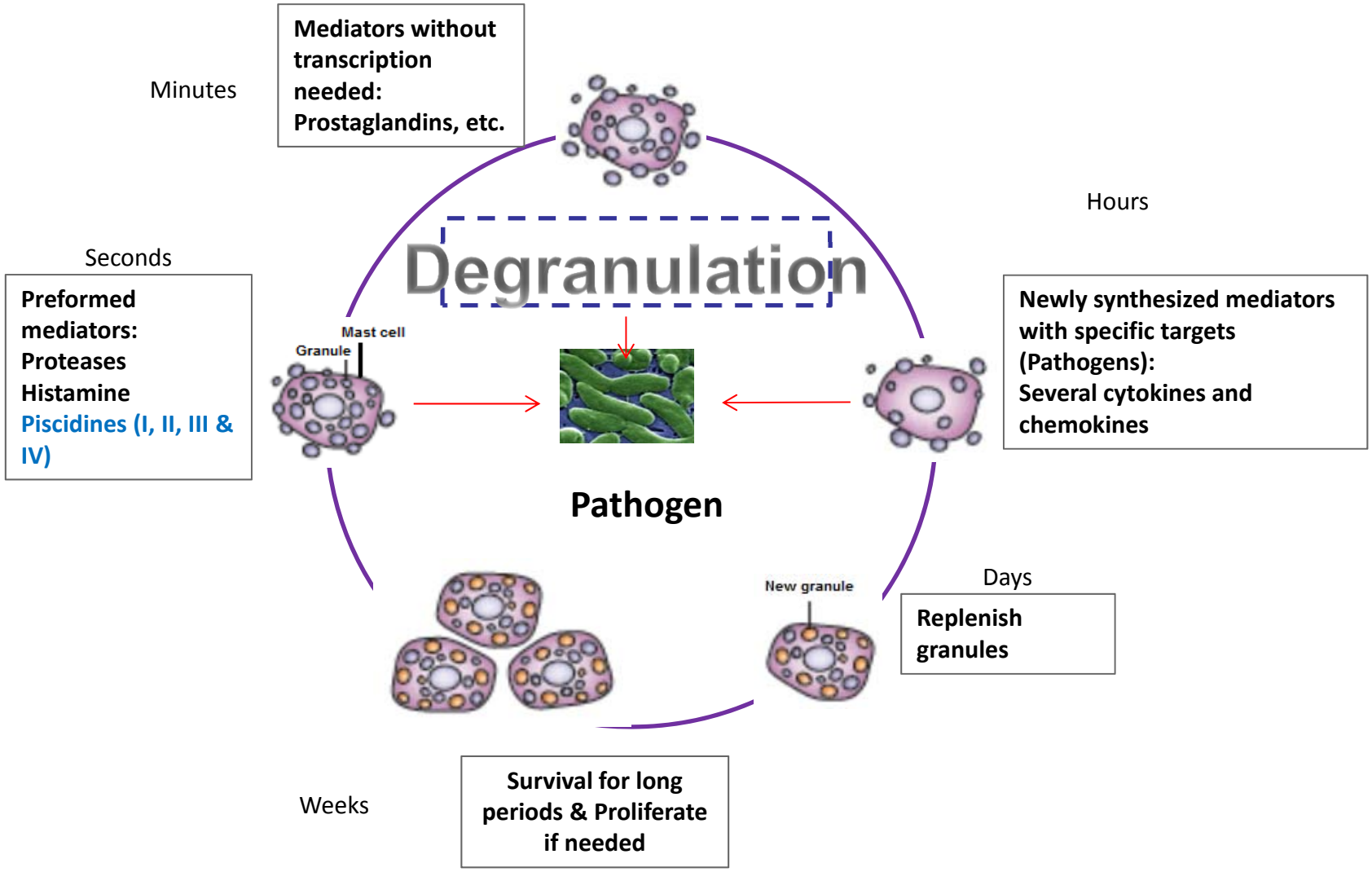
(Rebel et al., 2010).



Insiders of innate immunity in Teleost fish: The Mast Cells

Located the gill filaments and the intestinal submucosa layer

MCs functions in pathogen surveillance



Conclusions

- lack of appropriate markers to unequivocally identify, isolate and functionally characterize the different immune cell types present in different species (Mast cells)
- High diversity of TLR (why?)

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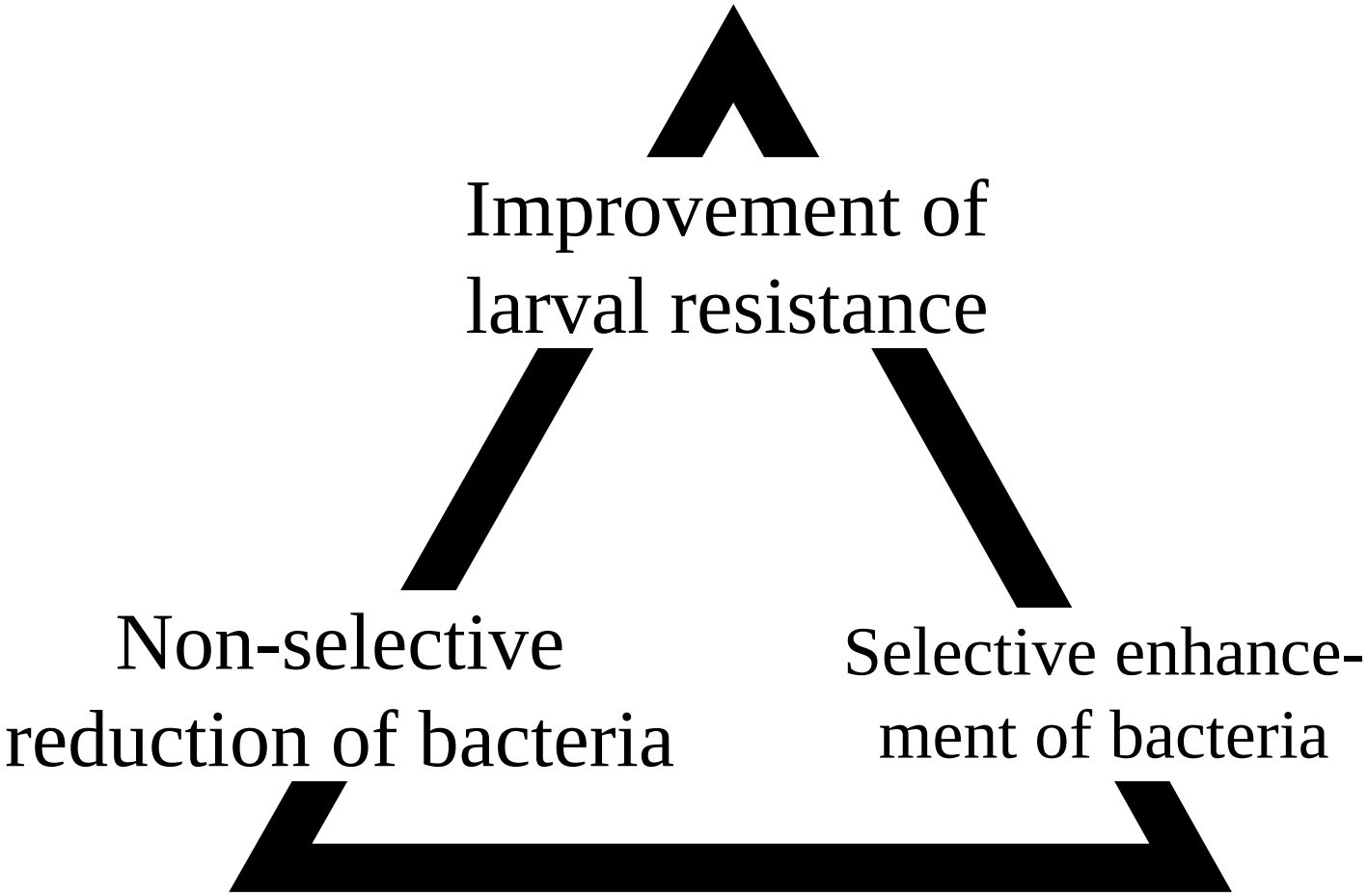
Three elements in a strategy for microbial control



Vadstein et al. 1993.

Methods for microbial control

Non-selective reduction of microbes:

- Surface disinfection of eggs
- Reduction in input of organic matter
- Removal of organic matter
- Grazer control of bacterial biomass

Selective enhancement of microbes:

- Selection for desirable bacteria
- Addition of selected bacteria to tanks
- Incorporation of selected bacteria in feed

Improvement of resistance against microbes:

- Stimulation of general immune system (beta glucan)
- Stimulation of specific immune system (vaccination)
- Modulation of general and specific maternal immunity
- Nutritional supplements improving susceptibility to microbes and wound healing

Vadstein et al. 1993 & 2004

Holistic approach

- In general there has been a bias to non-selective reduction with disinfection and selective enhancement and probiotics

- There is need for new thinking and diversification of approaches, most probably relying on *an appropriate yet to be defined mixture* of unselective reduction, selective enhancement and increasing resistance

THANKS