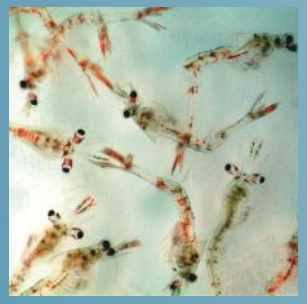
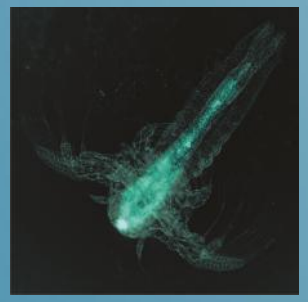
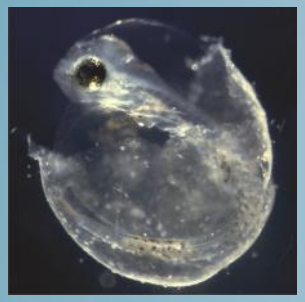
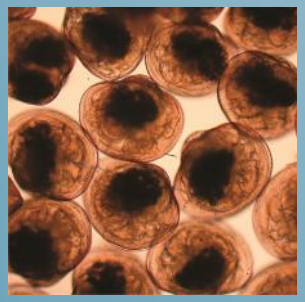




larvi 2013

6th fish & shellfish larviculture symposium

Lessons learned from gnotobiotic systems and the effect of bacteria on growth, survival and gene expression in marine larvae

Torunn Forberg

ghent university, belgium, 2-5 september 2013

LESSONS LEARNED FROM GNOTOBIOTIC SYSTEMS ON THE EFFECT OF BACTERIA ON GROWTH, SURVIVAL AND GENE EXPRESSION IN MARINE LARVAE.

Torunn Forberg and Olav Vadstein

NTNU
Norwegian University of Science and Technology

Contributions of bacteria to their vertebrate hosts

- Stimulate immune system- protect against infection
- Provide vitamins and extra metabolic capabilities
- Digestion and gut development

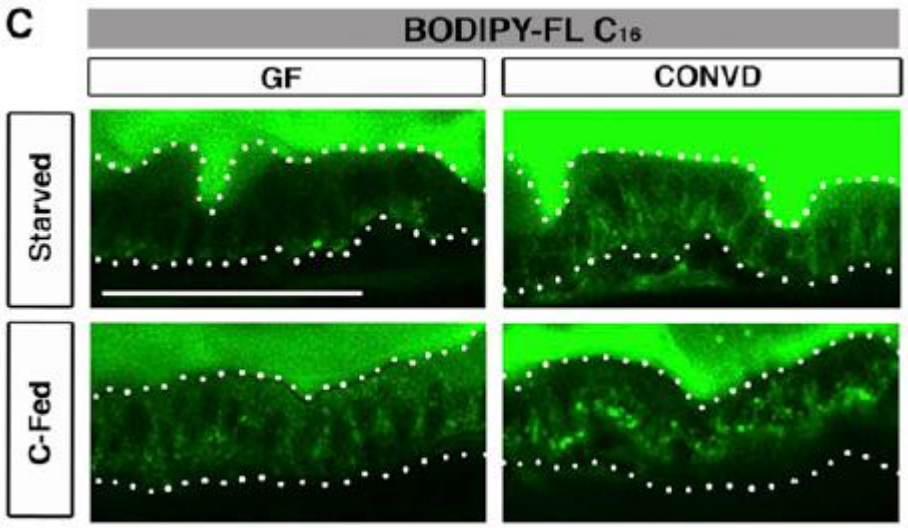
Gnotobiotic systems

- Conventional/xenic
- Microbiota is dynamic
- Gnotobiotic

-Germfree/axenic, mono/di/tri –xenic etc.

Gnotobiotic zebrafish (*Danio rerio)*

- 212 genes regulated by microbiota
- Reduced growth and gut development
- Impaired nutrient uptake



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Rawls et al (2004). PNAS (101:13 4596–4601)
Semova, et al (2012). Cell host & microbe (12:3 pp.277 - 288)

Gnotobiotic systems marine species

- Turbot (*Scophthalmus maximus*)
- Halibut (*Hippoglossus hippoglossus*)
- Sea bass (*Dicentrarchus labrax)*
- Cod (*Gadus morhua*)

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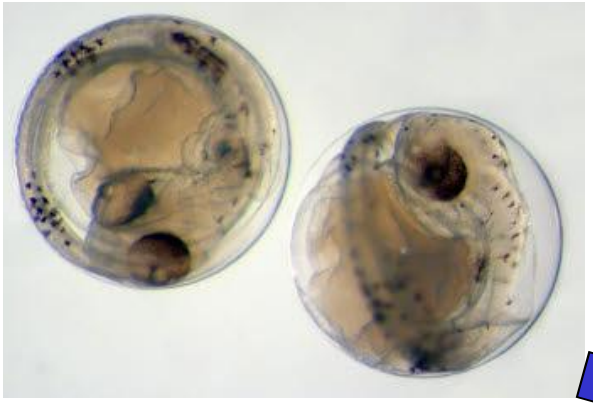
Munro, et al. (1995). *Applied and Environmental Microbiology* 61.12 (1995): 4425-4428.
Verner-Jeffreys, et al. (2003) *Diseases of aquatic organisms* 56.2 (2003): 105-113.

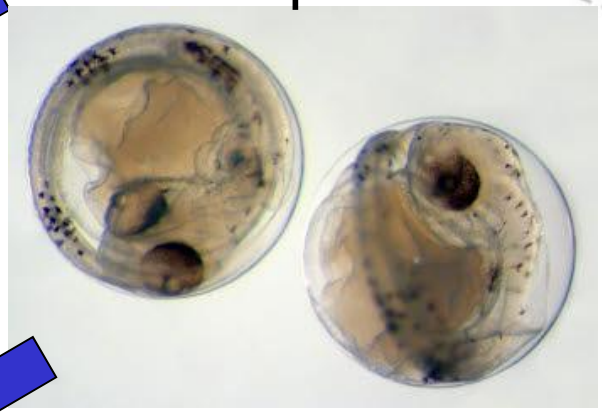


Sea bass and cod protocols

Glutaraldehyde

10 ppm rifampicin
and ampicillin

1x sea bass
2x cod



Reared with or without
antibiotics

Photo by Tora Bardal

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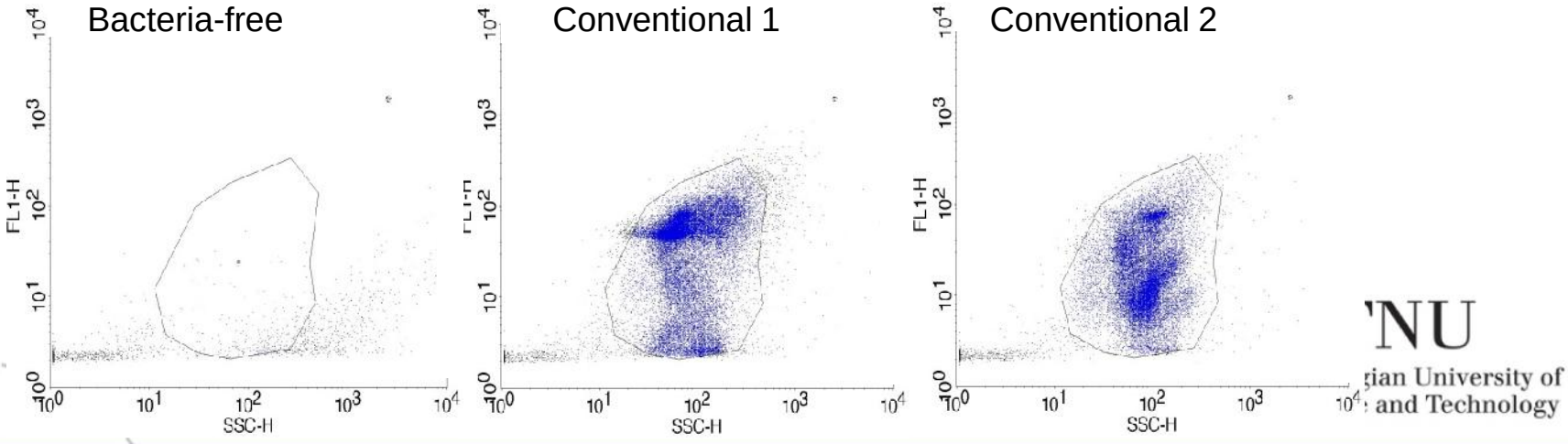
Dierckens et al. (2009) *Environmental Microbiology* 11:2 526-533.
Forberg et al. (2011) *Aquaculture* 315:3 222-227

Methods to confirm gnotobiotic state

- Culture based
- Amplification based
- “Direct counts” –microscopy or flow cytometry



Survival and growth

- Germ-free turbot, sea bass and cod- high survival
- No difference in growth detected in germ-free and conventional cod
- Germ-free sea bass larvae bigger and more developed digestive tract than conventional..
- Stressful conditions – Germ-free and conventional sea bass similar growth

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Rekecki et al (2009). *Aquaculture* 293:1-2 8-15

Gene expression

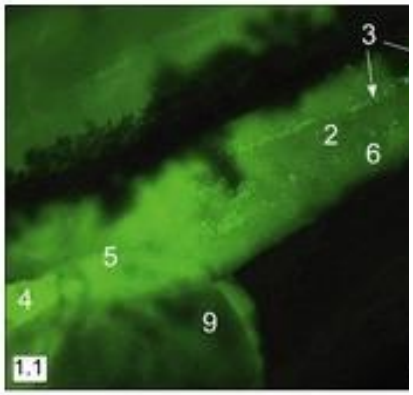
- Cod larvae: 14 putative host-response genes identified, involved in processes such as immune response, nutrient uptake and cell growth.

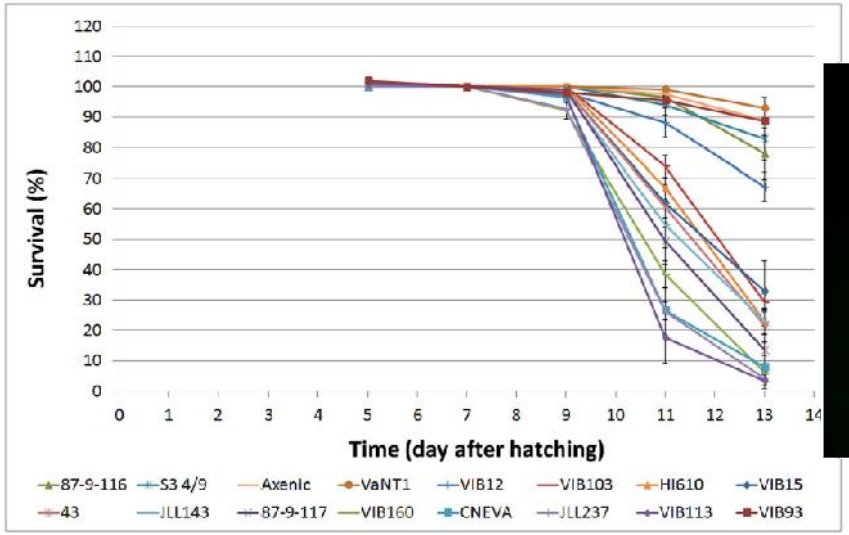
- Microbial specificity was observed both with regards to what bacteria were present and to the status (live/dead) of the bacteria.

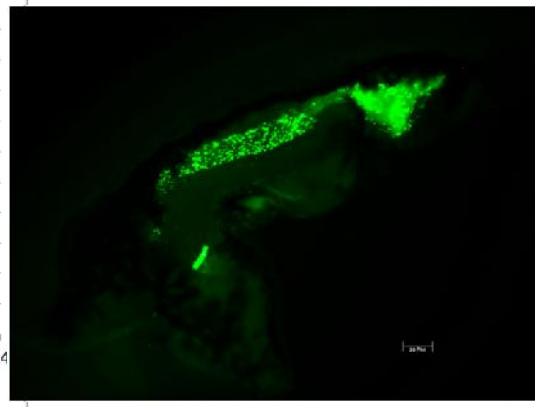
Forberg et al (2011). *Aquaculture Research* 42.5 664-676.
Forberg et al (2012). *Veterinary Microbiology* 157:3 420-427

Applications with monognotobiotic conditions

- Cod and probiotic candidate bacteria
- Sea bass & cod GFP-labelled *V.anguillarum*
- Sea bass virulence of *V.anguillarum* serotypes and mutants







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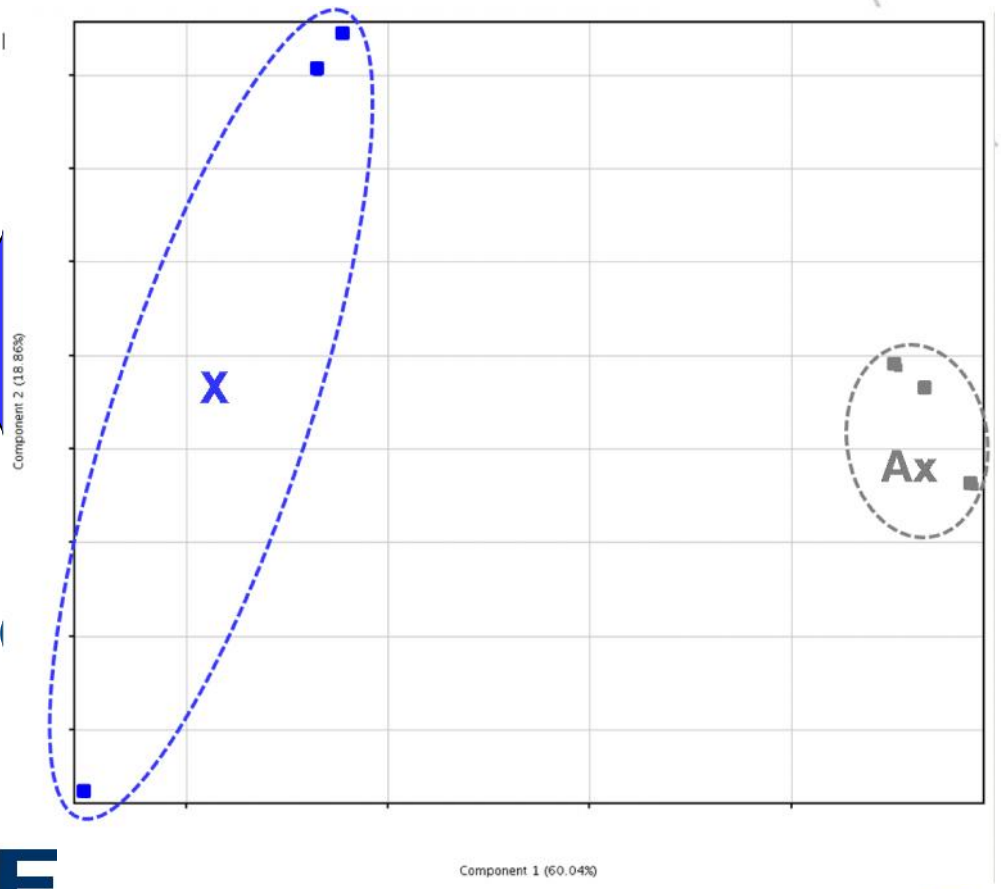
Rekecki, et al (2012)."*Journal of fish diseases* 35.4 265-273.
Frans, et al. (2013) *Agricultural Sciences* 4.6A (2013): 30-38.

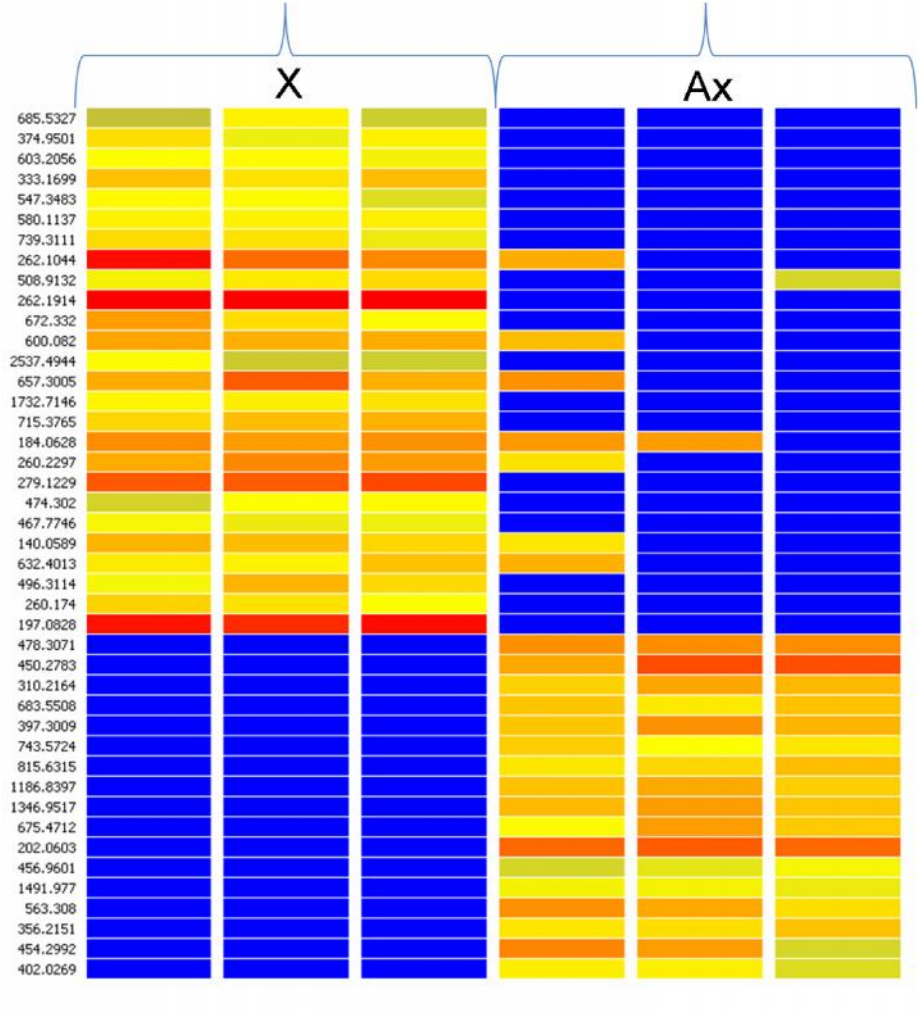
Germ-free vs conventional cod metabolome



Numb

Aasen et al.

Axenic *vs* xenic cod larvae



"Heat-map", most significant compounds

Identification of significant compounds

Comp.	Xenic	Axenic
Phospholipids	-	+
Lyso-PLs	+	-
Conj. bile acids	-	+
Di-/tripeptides	+	-
Modified aa	+	-
Flavonoids	+	-

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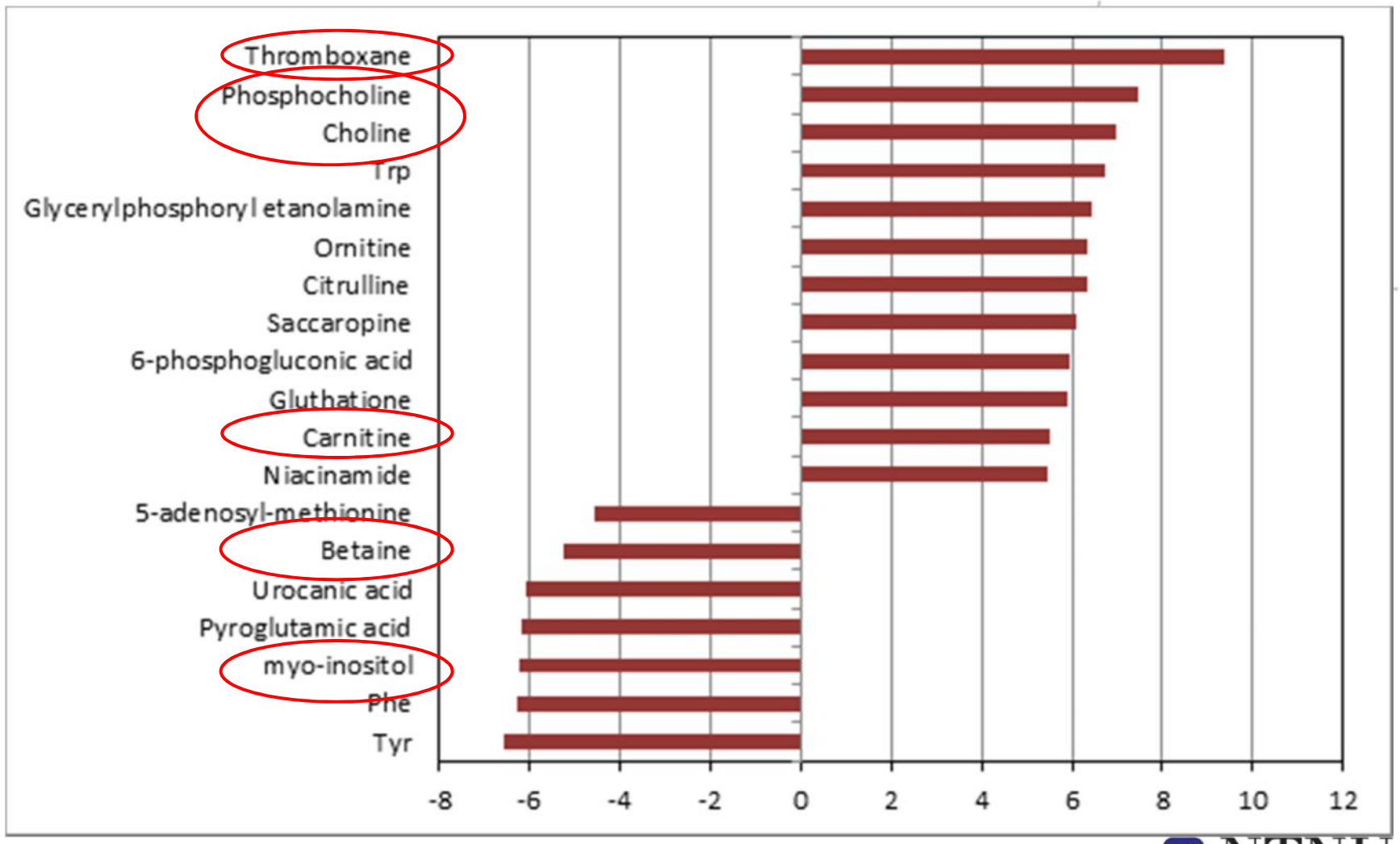

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Conclusions & perspectives

- Both cod and seabass represent robust gnotobiotic systems for marine larvae
- Effects of germfree state seem to differ from zebrafish with regards to growth
- Some host-response genes identified in cod, next stage - high throughput methods
- Metabolome analysis confirms the many effects of commensal microbiota on host metabolism.
- More studies needed!

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