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The intestinal microbiota in rainbow trout is influenced by diet type and *Yersinia ruckeri* challenge

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INTRODUCTION

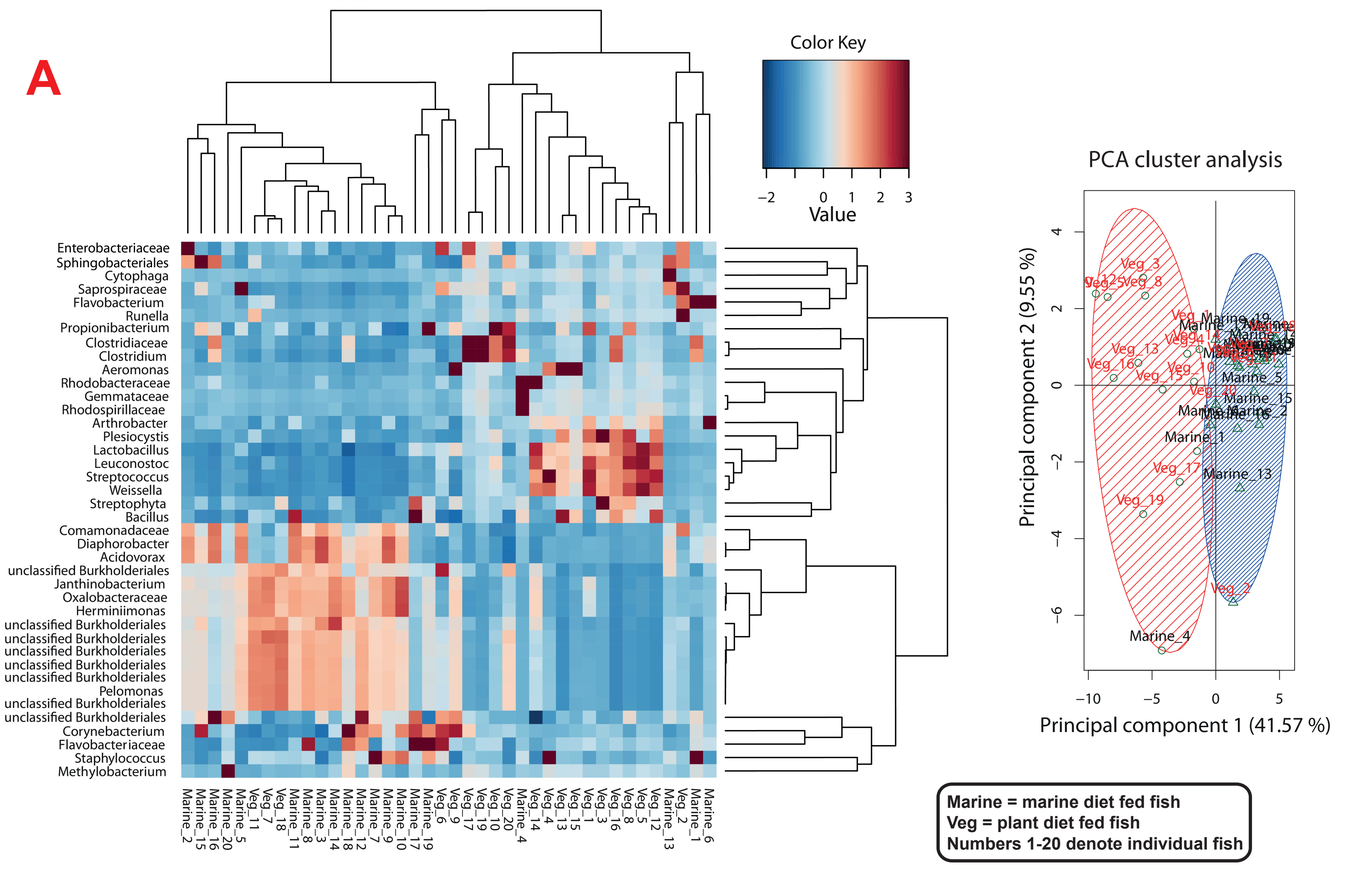
Due to world-wide limitations of marine fish meal and oil, plant ingredients such as pea and rape-seed may be used as substitutes in the production of aquaculture fish feed.
Previous studies from warm-blooded animals show that the gut microbiota community profile is dependent on the diet type and that it also cross-talk with the immune system. Further, infection may also affect the community structure. In this study these subjects were studied in rainbow trout (*Oncorhynchus mykiss*).

OBJECTIVES

The objectives of the studies were **i)** to examine how the microbiota in the intestine of rainbow trout is influenced by two different diet types with either a marine-based or plant based origin by the use of Illumina HiSeq sequencing; **ii)** to study if an experimental bath infection by *Yersinia ruckeri* influences the intestinal microbiota community and **iii)** to examine the immune response in the two different diet treatments groups after challenge with *Yersinia ruckeri*.

Influence of diet type on intestinal bacterial community (non-infected fish)

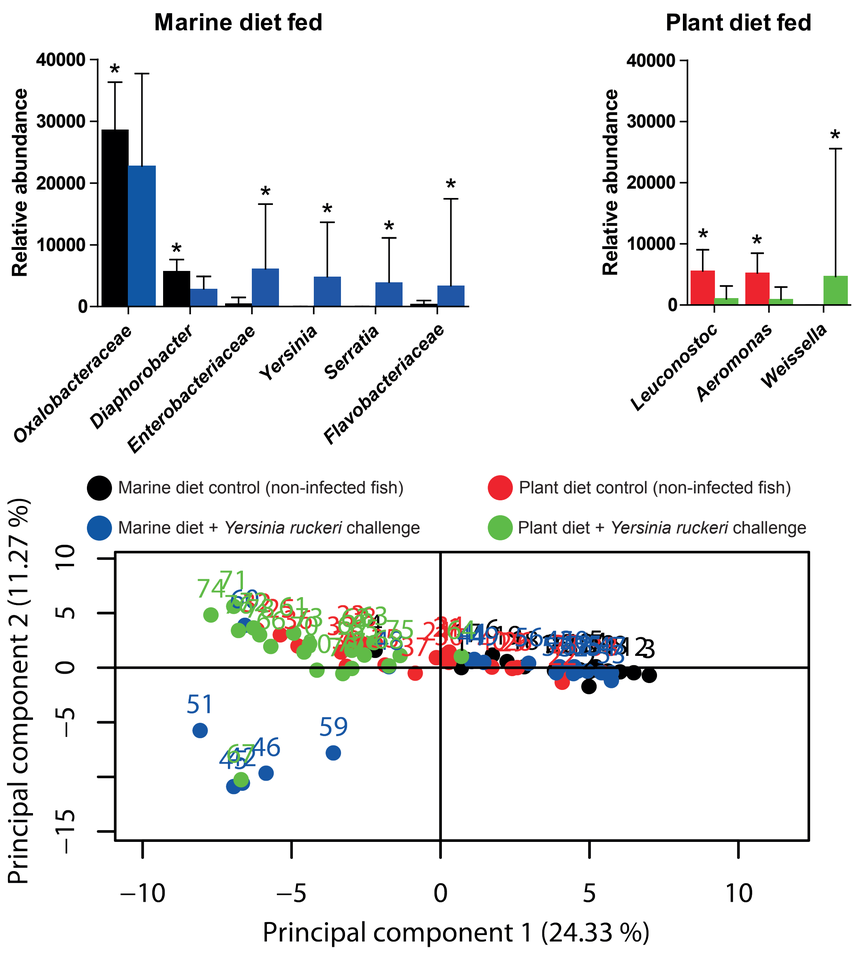
A



Marine = marine diet fed fish
Veg = plant diet fed fish
Numbers 1-20 denote individual fish

Influence of *Y. ruckeri* on microbiota and gene transcription

B



C

Regulation of transcription level (*Y. ruckeri* challenged minus control fish)

Diet /gene	Mbl 1	Mbl 3	C3	C5	IL-1β	CD8	Foxp3a	Foxp3b	IgT
Marine					↑				↓
Plant	↓	↓	↓	↓		↓	↓	↓	↓

RESULTS

Healthy fish fed the plant diet had a higher abundance of bacteria from the genera *Lactobacillus*, *Leuconostoc*, *Streptococcus* and *Weissella* relative to fish fed the marine diet. The intestines of marine fed fish had high abundances of bacteria from phylum Proteobacteria relative to the plant diet groups (Fig. A).
Yersinia ruckeri gave rise to significant changes in abundance of 9 different taxons, but these differed dependent on the diet type (Fig B). Fish number 42, 45, 46, 51, 59 (blue) and 67(green) on the PCA plot had a high load of *Yersinia*-specific 16S rDNA.
The immune response (challenged versus non-infected control fish) differed dependent on the diet type and showed a down-regulation in challenged fish fed the plant diet, whereas there was a pro-inflammatory response in fish fed the marine diet (Fig. C).

CONCLUSIONS

Diet type had a high impact on the intestinal microbial community in healthy fish.
A smaller influence was seen after bath infection by *Yersinia ruckeri*, changing the relative abundance of a few genera compared to non-infected control fish. The differences were also dependent on the type of diet.
The gene transcription data revealed that most of the examined genes were down-regulated in challenged plant diet fed fish, whereas a pro-inflammatory response occurred in challenged fish fed the marine diet. Thus, the diet has a high impact on the gene transcription, but the microbiota may also be involved in this regulation.

MATERIALS & METHODS

Rainbow trout (mean size of 4 g) had been fed either a commercial diet of marine origin (Inicio, Biomar A/S) or tailor-made diet containing plant parts; rapeseed oil (instead of fish oil as in Inicio) plus pea proteins since first feeding. Subgroups of these fish were experimentally bath infected by *Yersinia ruckeri* serotype O1. Five days post challenge the intestines were collected from control fish and infected fish (*n*=20 per diet type) for characterisation of the microbial community and for measurement of immune gene transcription. The microbiota was characterised on basis of the V5 region of the 16S rRNA gene by Illumina HiSeq sequencing. Sequences were analysed by the open-source software package BION-meta and taxonomically classified according to the Greengene database (for further information see http://box.com/bion). Immune gene transcription was measured by qRT-PCR on the Stratagene MX3000P real-time PCR platform.

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