

“Characterization of the variability and stability of the gastrointestinal microbial community (MC) of *Oreochromis niloticus* larvae, reared in active suspension systems for 42 days from the moment of first feeding”



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Introduction



The density of microbiota in aquaculture systems is higher than in natural environments!

Bacteria key role: host **health** and **performance**

Interest in controlling the gut **microbial community** to benefit the host

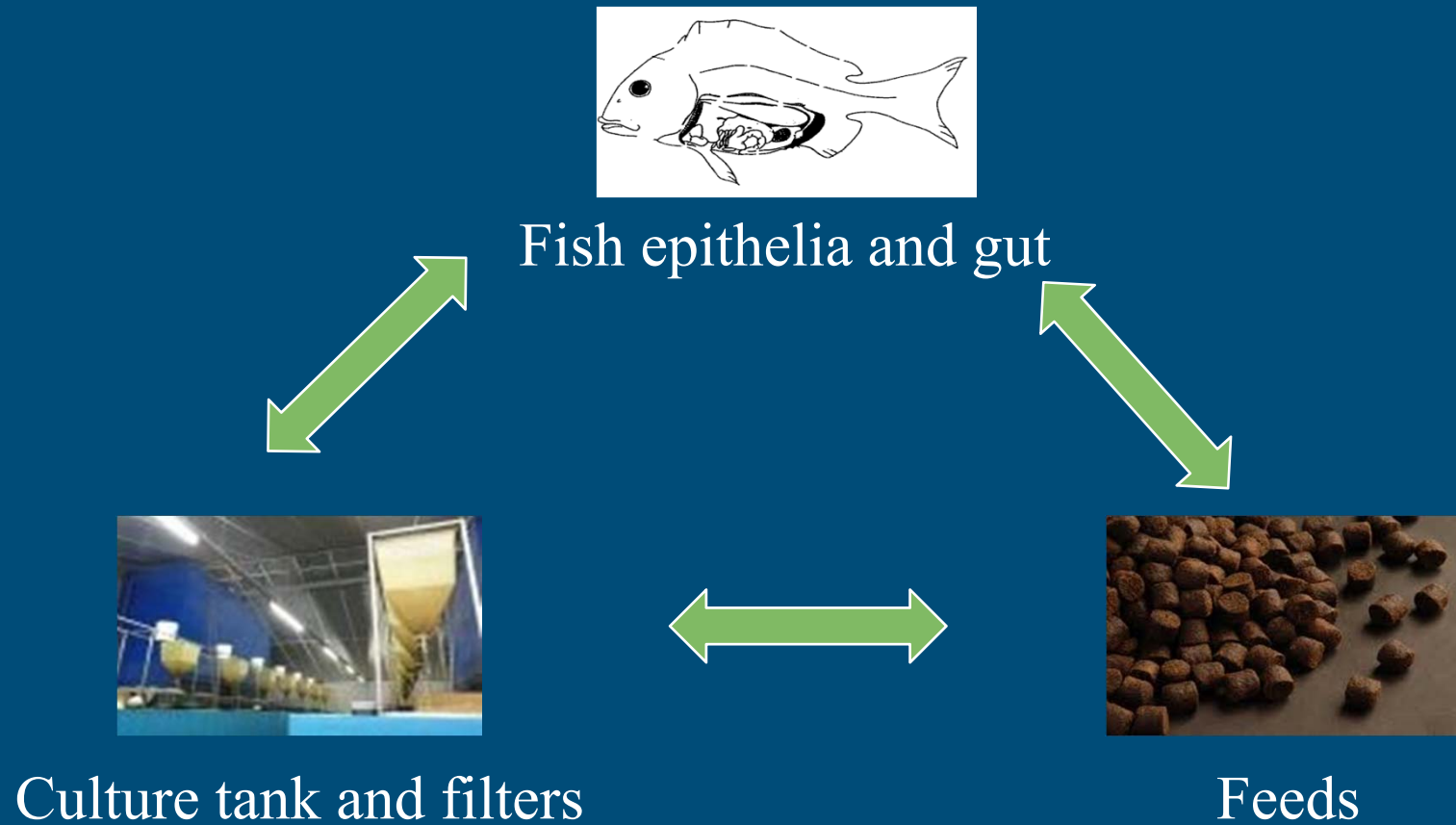
Variation among individual gut-MC?

Different factors (host, microbe, environment, genetic factors)



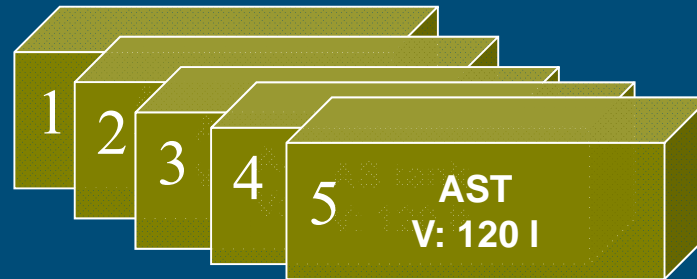
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Microbiota in aquaculture systems

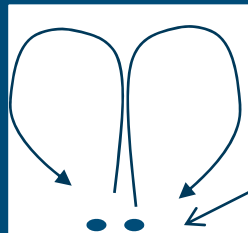


Research set-up and goals

5 active suspension tanks
Stocking: 100 fish per tank



1 batch larvae
Same water



Zero water exchange
Porous aeration pipes

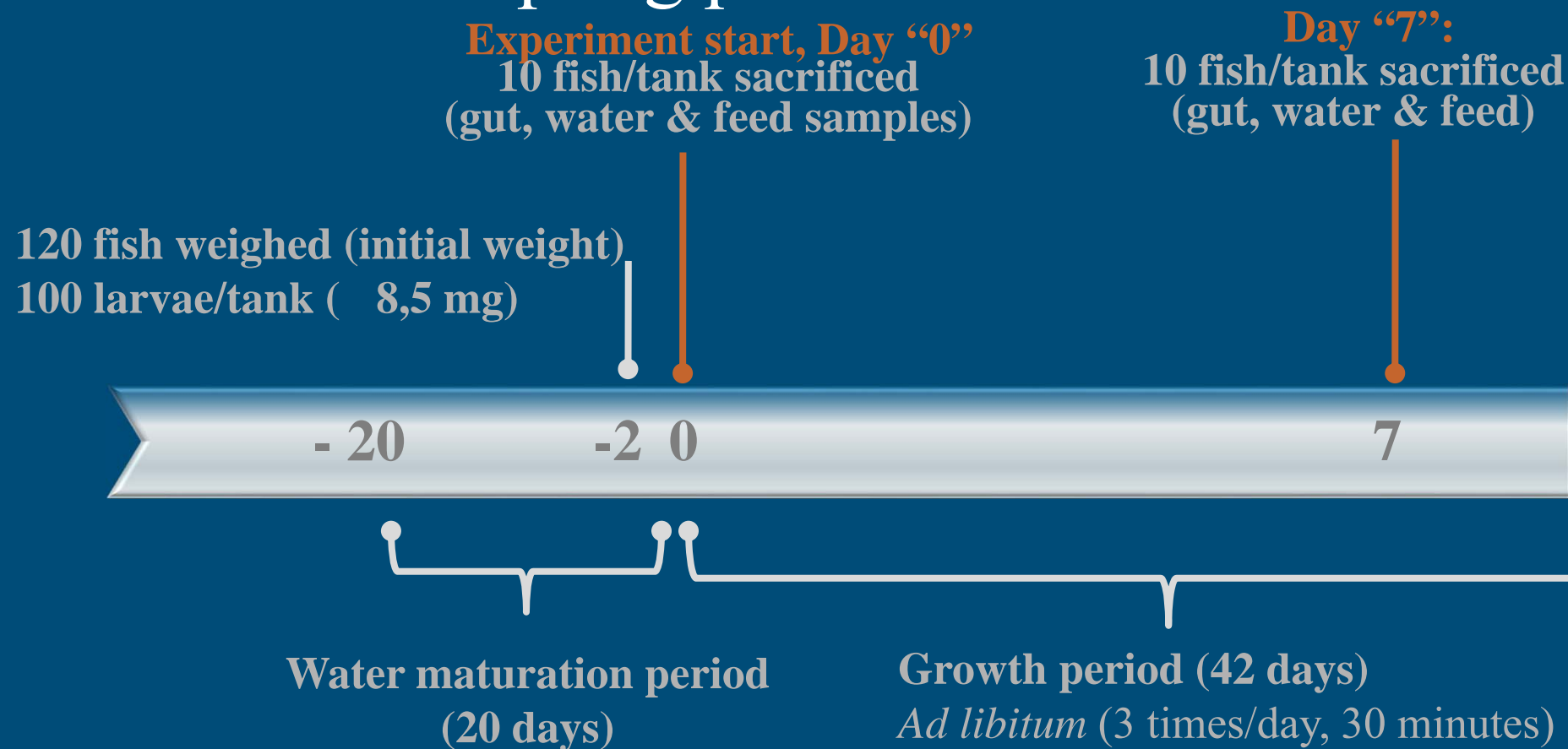


Objectives:

- Determine the variation **between gut-MC and water-MC (tank)**
- Monitor the **evolution (stability?) of gut-MC and water-MC over time**



Time line – Sampling points



Time line – Sampling points

Day “14”

10 fish/tank sacrificed per sampling point (gut, water & feed samples)

Day “28”

End of experiment Day “42”

Final weight measured
50 larvae/tank proximate analysis

14

28

42

Growth period (42 days)

Ad libitum (3 times/day, 30 minutes)

Water measurements (O₂, pH, T, TAN, NO₂, NO₃, CO₂, PO₄, urea)

Water treatment with O₃ + UV



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Data collection

- Per sampling day;
 - 3 fish guts per tank for PCR-PGGE
 - 1 water sample per tank for PCR-DGGE
 - 1 sample of feed for PCR-DGGE
- Daily water quality monitoring



➤ Microbiological Analysis



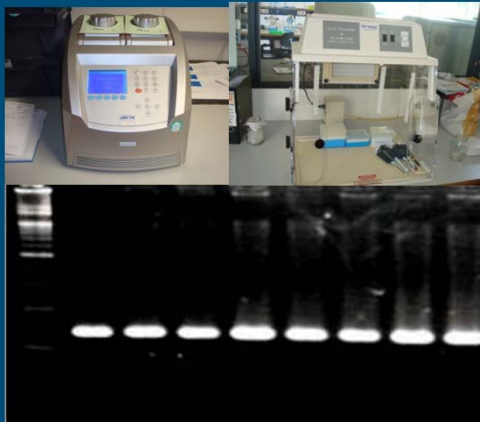
Water
Gut
feed
→



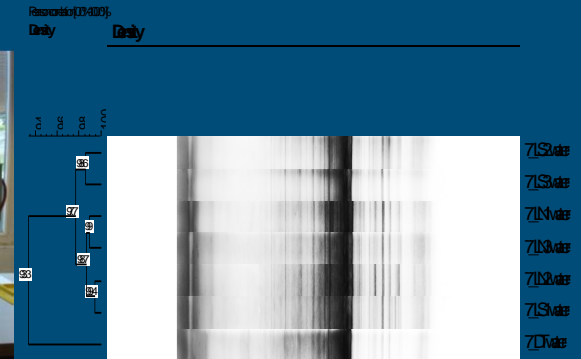
DNA
extraction



PCR



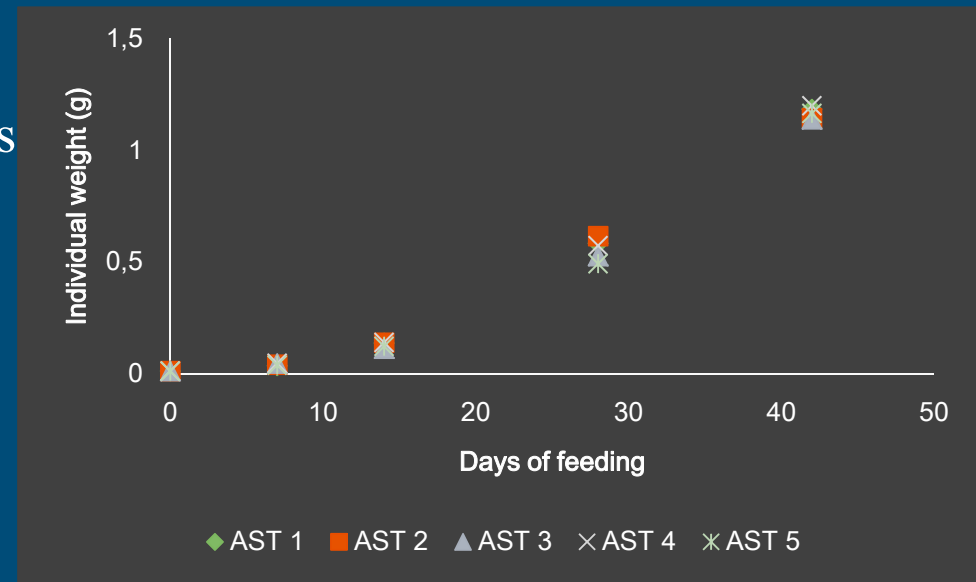
DGGE



Results – Water quality & fish growth

- Water quality – excellent during whole experiment
- Good control on TAN and NO₂-N
- Fish performed and grew very good
- small differences between replicate tanks

11% bw day⁻¹; 0.7 FCR



DGGE gels

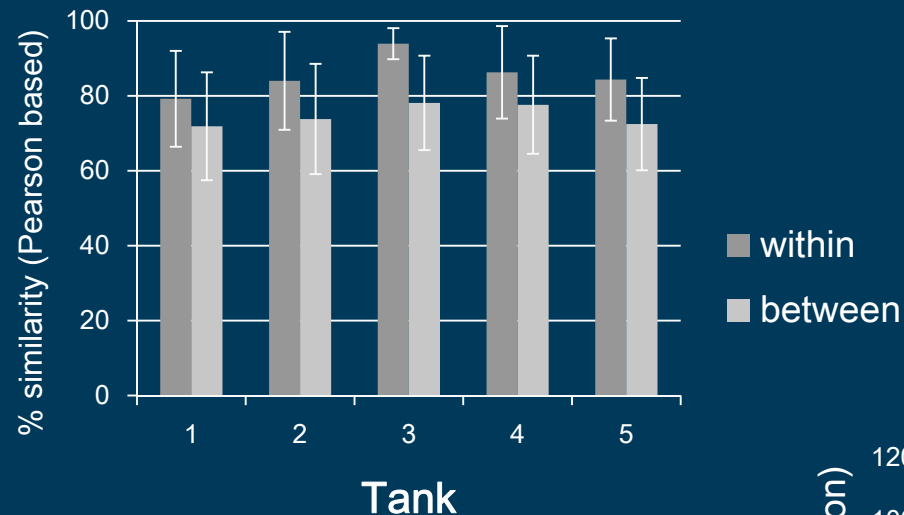


If possible: compared samples loaded on one gel

If not: controlled and aligned; $\Delta < 10\%$



Similarity gut-MC of individuals within and between tanks

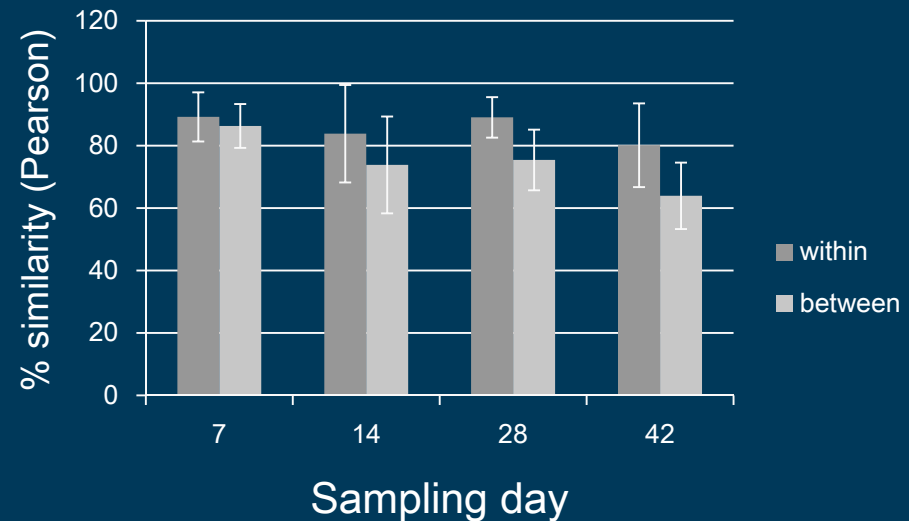


Tank comparison

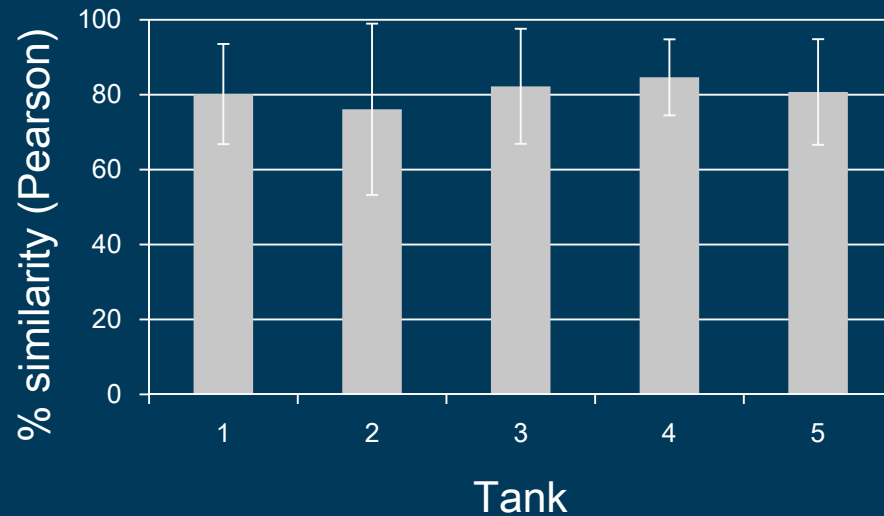
- 70-95% similarity;
- No significant difference 'within' and 'between' tanks

Sampling day comparison

- Within tanks; no decrease over time
- Between tanks, % similarity reduces over time

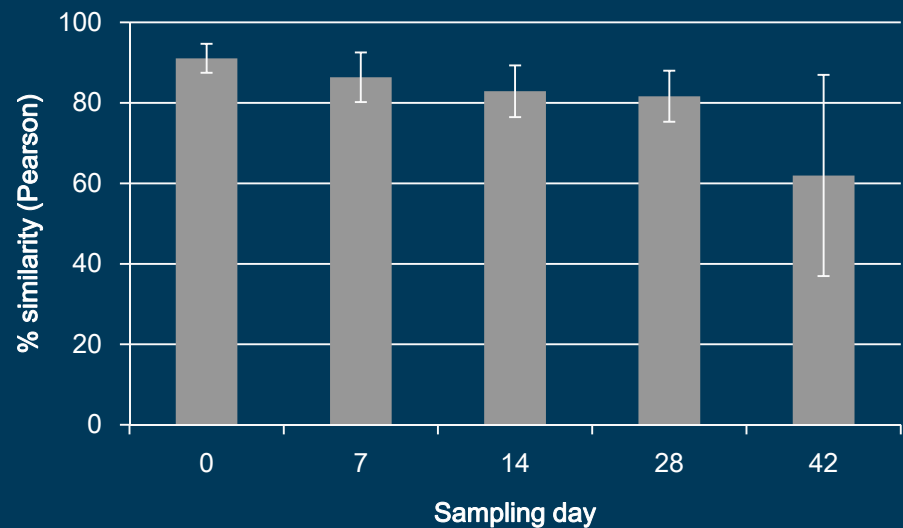


Water-MC similarities

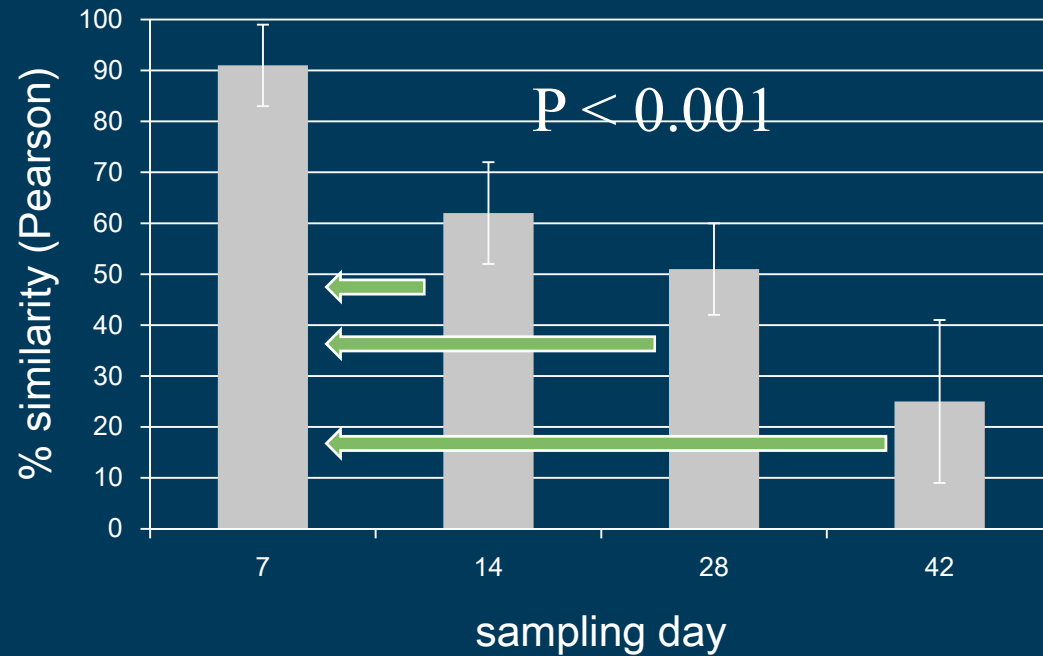


75 – 85% similarity
No differences between tanks

Trend of decrease over time,
From > 90 → 63% similarity



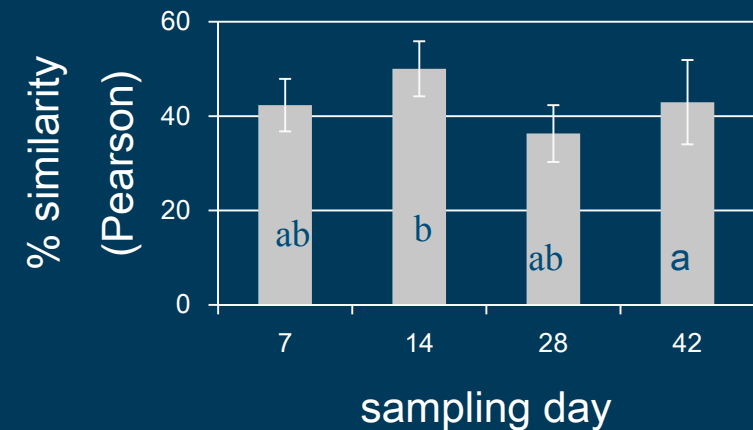
Gut-MC changes over time



Moving window analysis

Water over time

In water, % similarity fluctuates



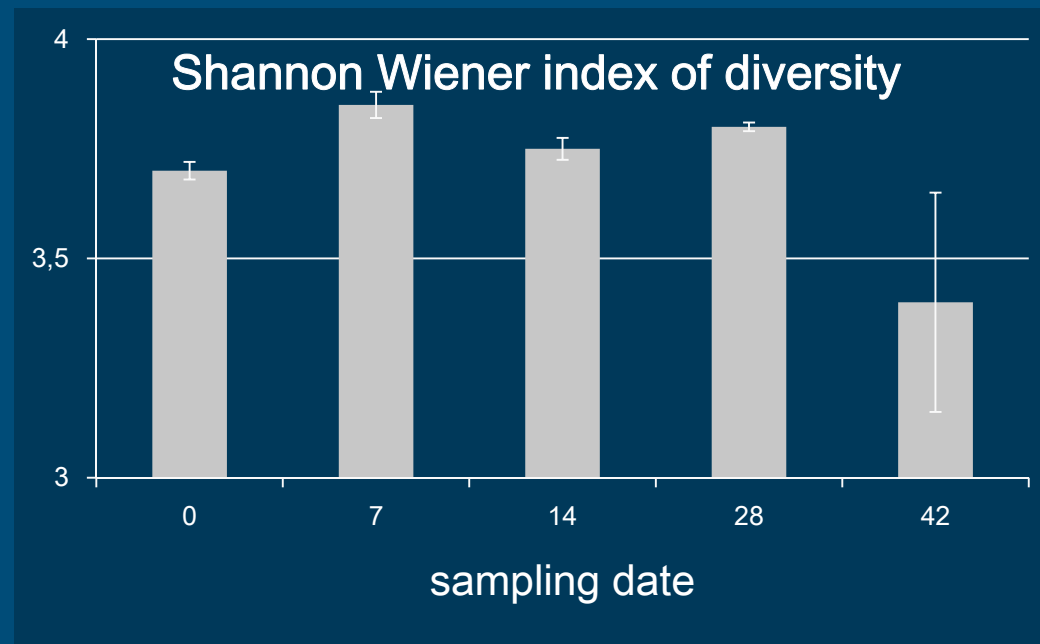
Shannon Wiener Index (H')

Indicator of complexity of ecological data. Measures weight of each species in the bacterial community. Infers on patterns of dominance.

Diversity in water:

Day 42:

More dominant species

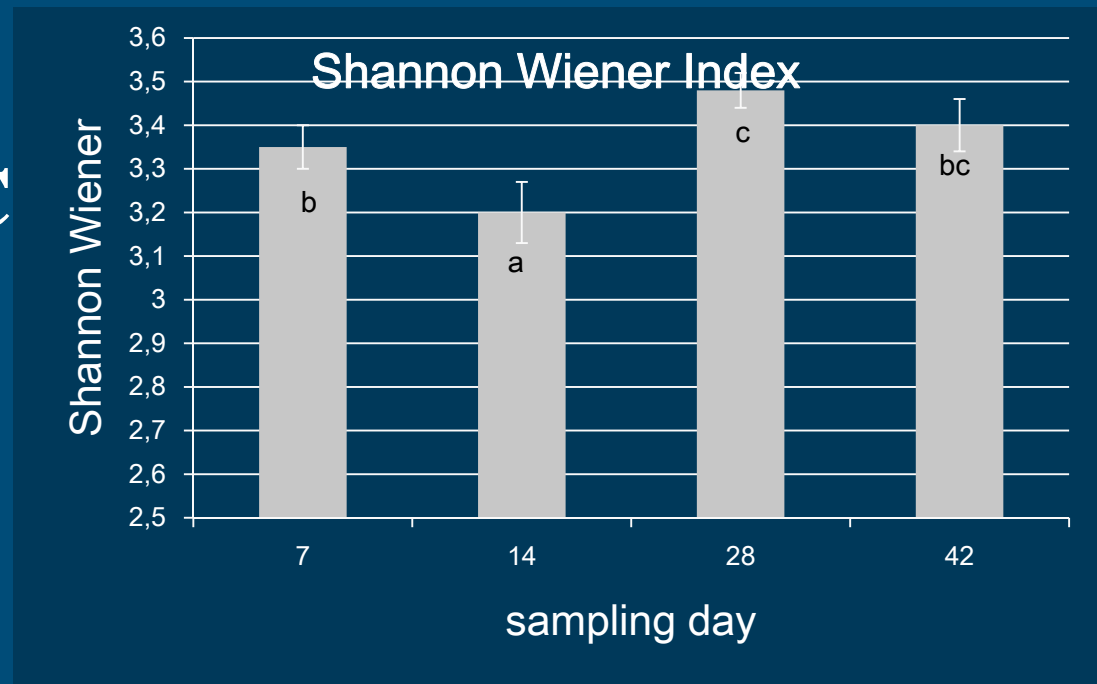


Shannon Wiener Index

Diversity in gut-MC

Variation over time

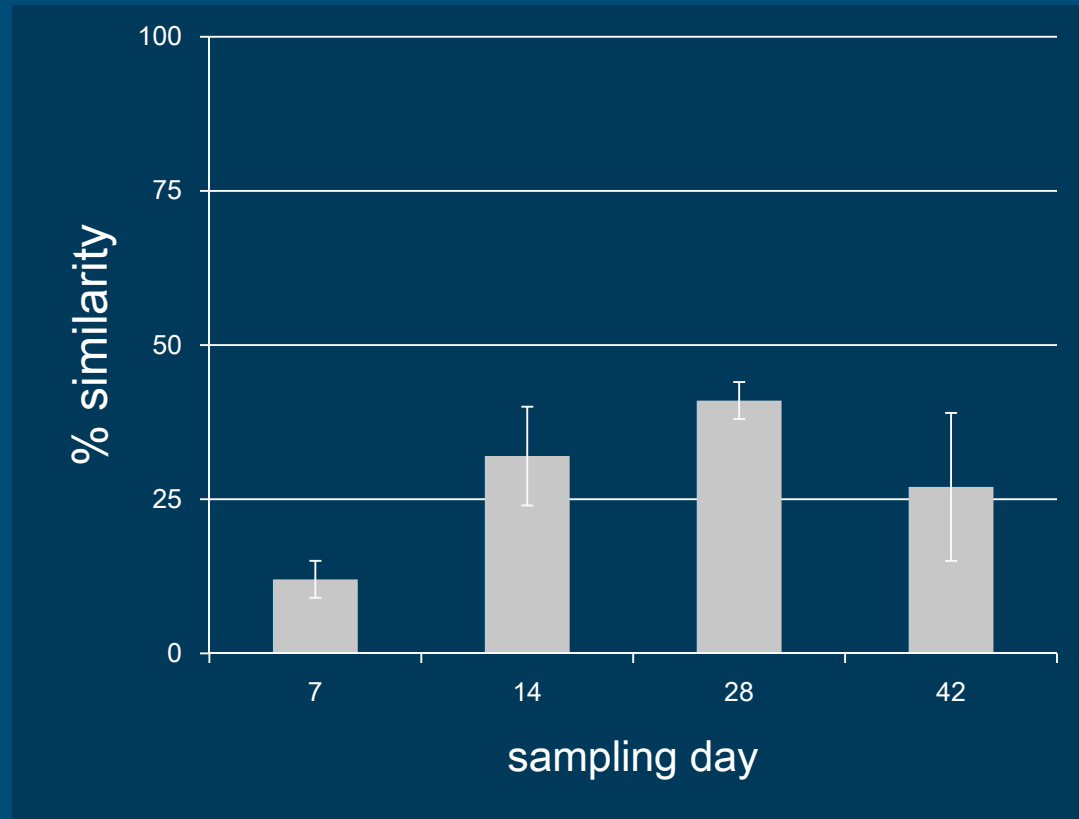
Highest evenness on day 28



% similarity water-MC & gut-MC

Low overlap between water-MC and gut-MC

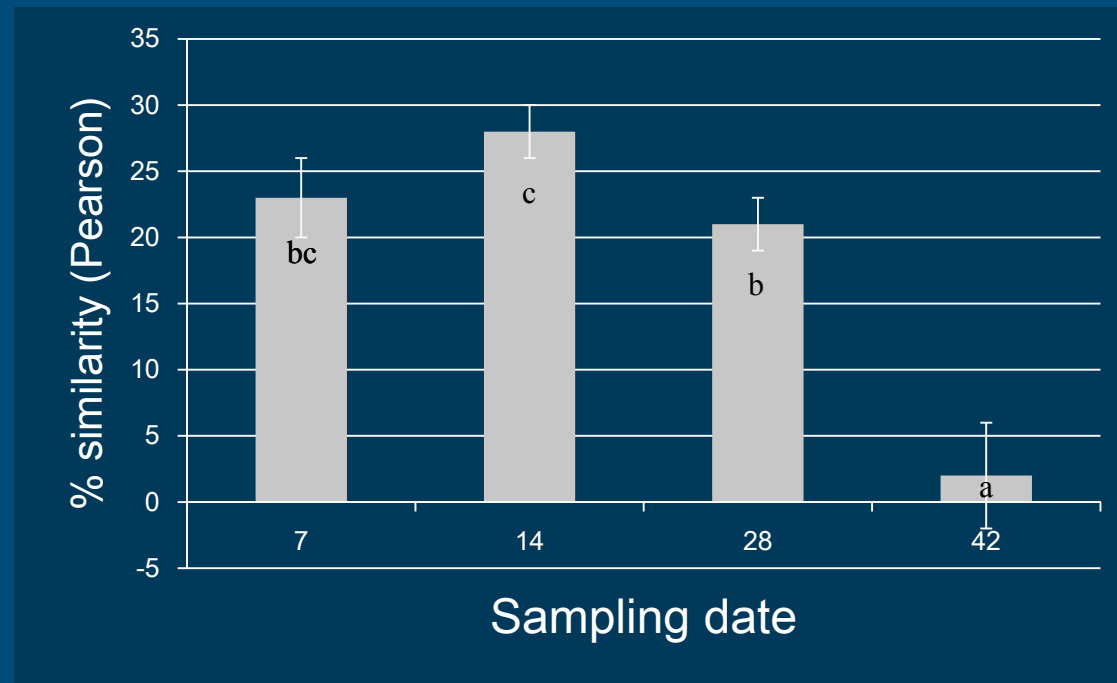
12 – 41%



% similarity between feed-MC & gut-MC

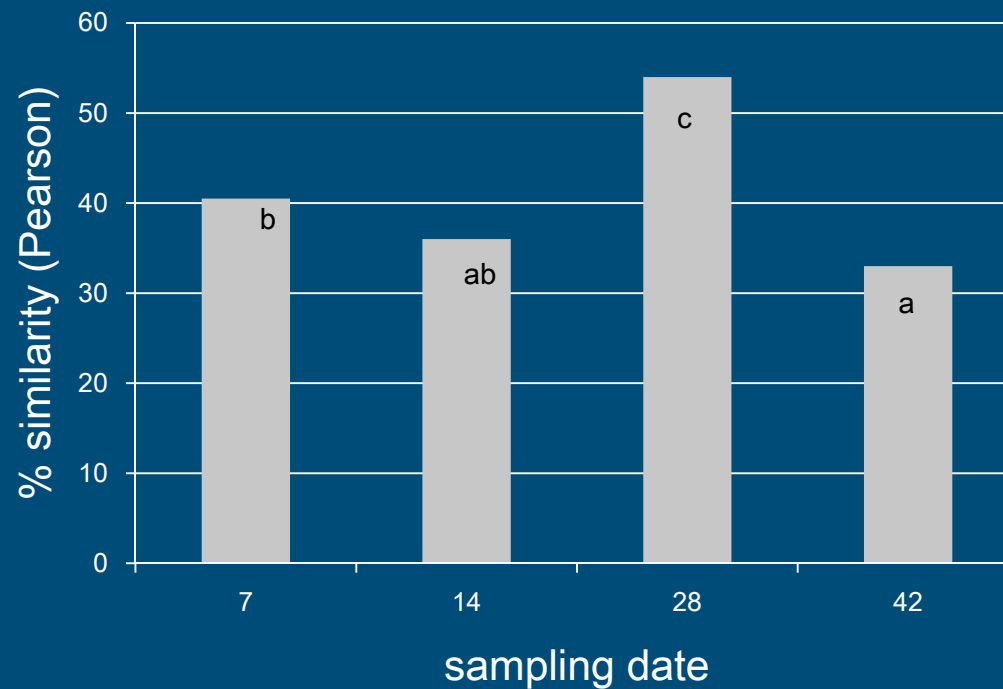
% similarity is low
(20 – 30%);

Very small on day 42



% similarity water-MC and feed-MC

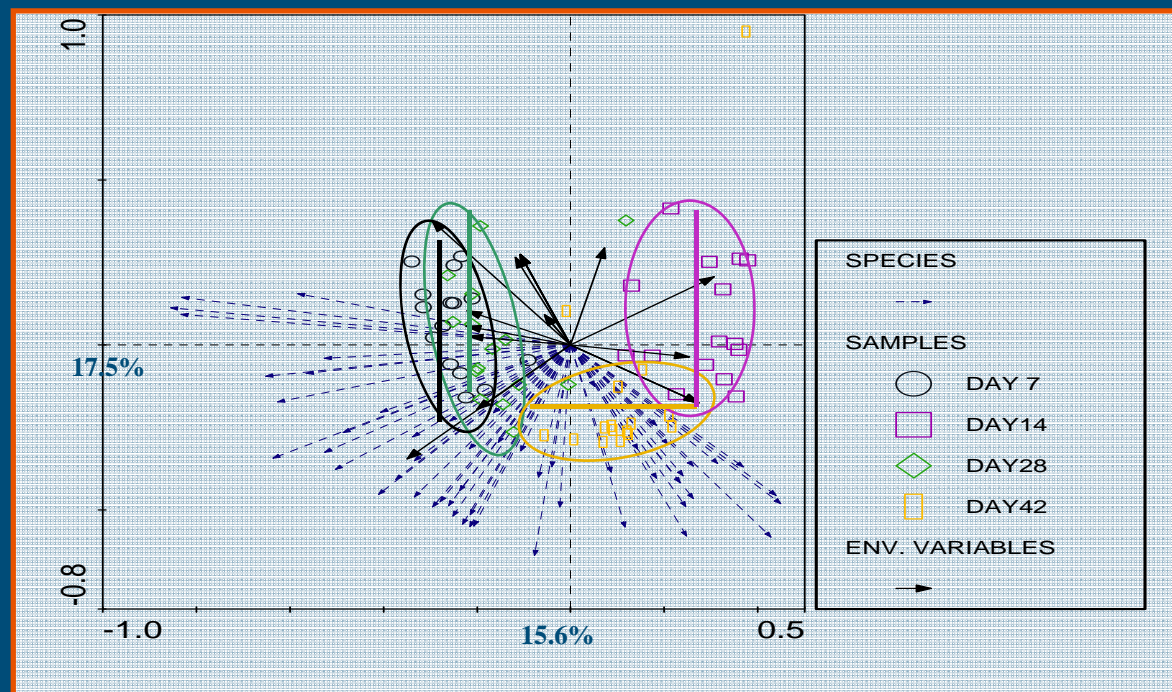
Highest variation on
day 28



Correlation of water-MC and gut-MC over time

Species (absence-presence)

Principal component analysis



**PCA – explained low % of
gut-MC variation (<33%)**

Representation of the first two PC generated from gathered absence-presence data of DGGE profiles, pertaining to gut MC from systems. The percentages provided at the first two canonical axes shown in the graph indicate the degree at which the PC, pertaining to the axis, is able to explain the variability observed in the data.

Conclusions

- Water quality stayed within limits favourable for fish health and growth.
- Tilapia grew to an average weight of 1.2 g, in 42 days (SGR 11 % bw/day; FCR 0.7) showing no differences between replicates ($P > 0.05$).
- On day 42, the DGGE profile of water-MC was more variable between active suspension tanks compared to the beginning.



Conclusions (continued)

- Initial high correlation between the gut-MC and feed-MC was apparent until day 14, but nearly disappeared on day 42.
- Water-MC and gut-MC show low % similarities on all sampling dates.
- The active suspension tanks explain the largest part of the variability in gut-MC of individual fishes.
- Sequencing to identify species next step



Thank you for your attention

Questions?



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