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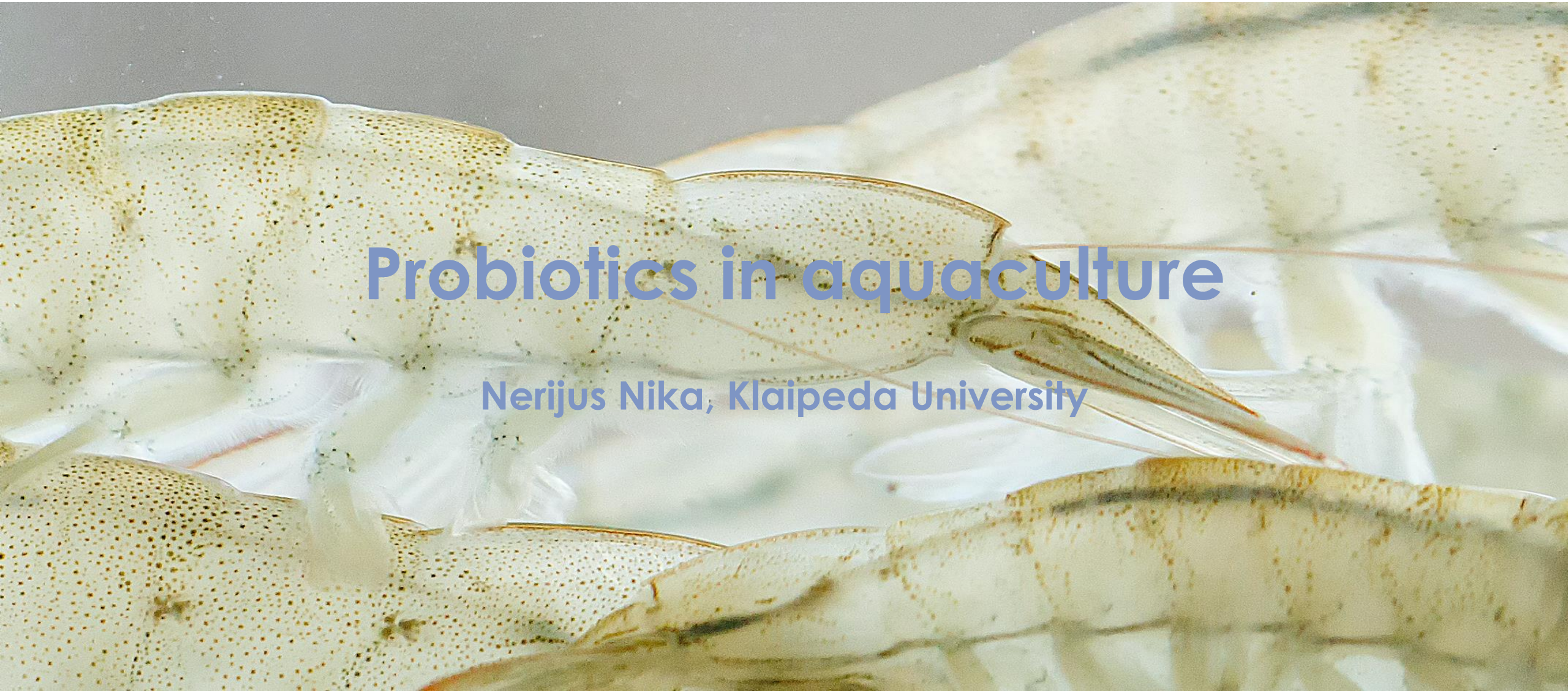
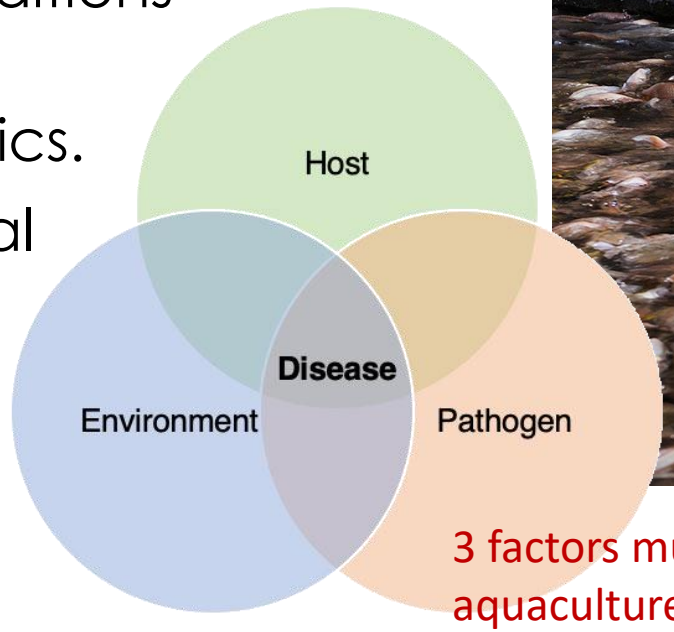


Probiotics in aquaculture

Nerijus Nika, Klaipeda University

Introduction

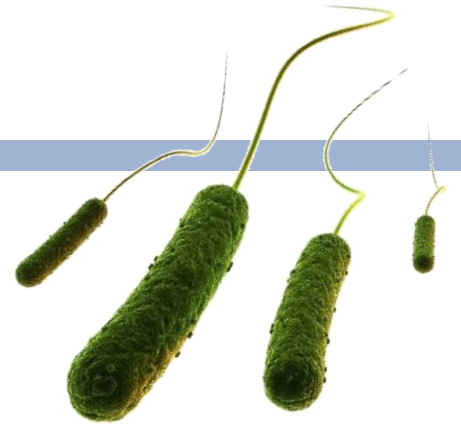
- In large-scale aquaculture, where aquatic animals are exposed to stressful conditions, problems related to diseases and deterioration of environmental conditions often occur.
- Problem of antibiotics.
- One of the potential solutions for the above mentioned problems are **probiotics.**



3 factors must be considered in how probiotics in aquaculture can help manage heath and avoid disease

What are probiotics?

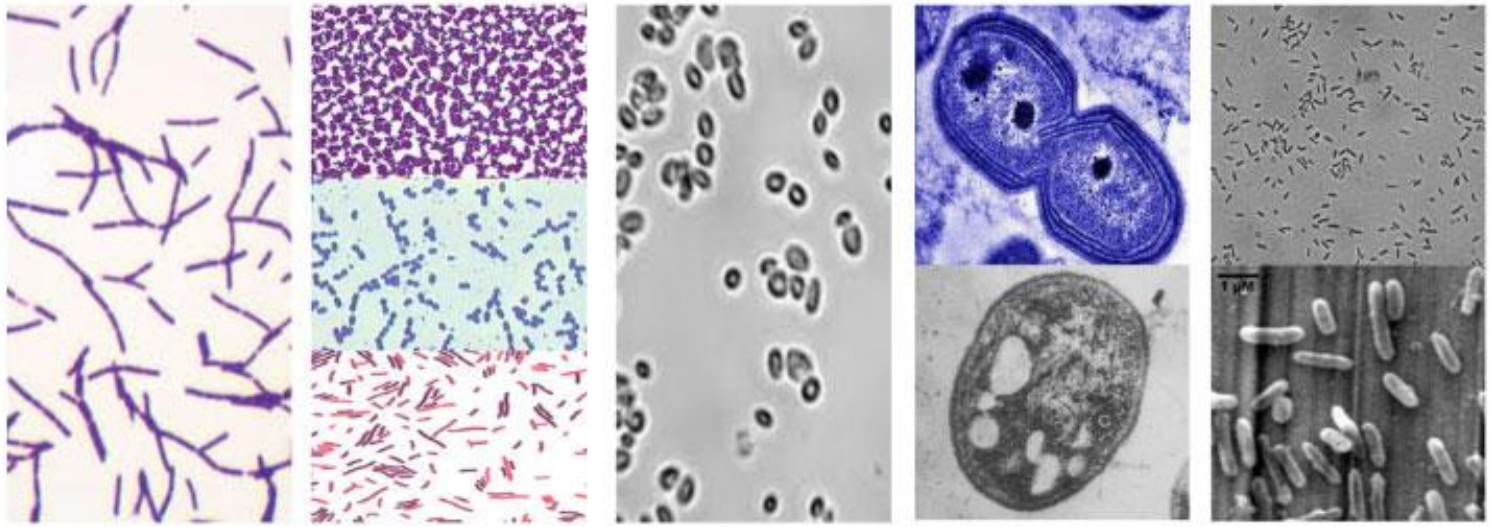
- **probiotics** are live beneficial microorganisms that, when administered in adequate amounts, improve a health benefit on the host



Gram-positive bacteria	Gram-negative bacteria
Arthrobacter	*Aeromonas*
Bacillus	*Agarivorans*
Brevibacillus	*Alteromonas*
Brochothrix	*Bdellovibrio*
Clostridium butyricum	*Burkholderia*
Carnobacterium	*Citrobacter*
Enterococcus	*Enterobacter*
Kocuria	*Neptunomonas*
Lactobacillus	*Phaeobacter*
Lactococcus	*Pseudoalteromonas*
Leuconostoc	*Pseudomonas*
Microbacterium	*Rhodopseudomonas*
Micrococcus	*Roseobacter*
Pediococcus acidilactici	*Shewanella*
Rhodococcus	*Synechococcus*
Streptococcus	*Thalassobacter*
Streptomyces	*Vibrio*
Vagococcus	*Zooshikella*
Weissella	

Source: Newaj-Fyzul et al. / Aquaculture 431 (2014) 1–11

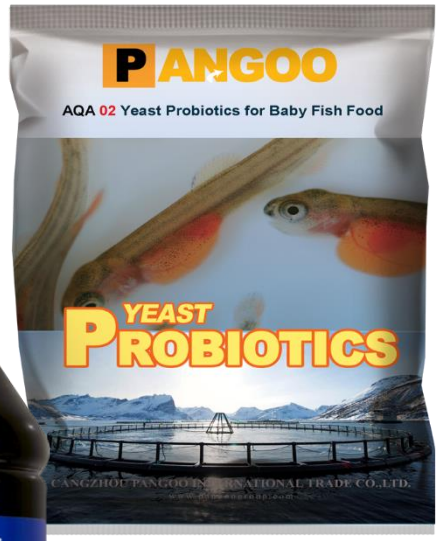
+Bifidobacterium, Flavobacterium, Debaryomyces……

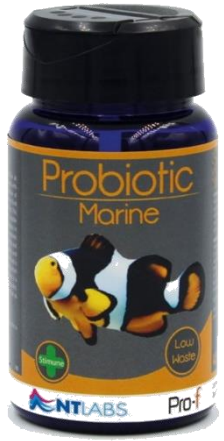


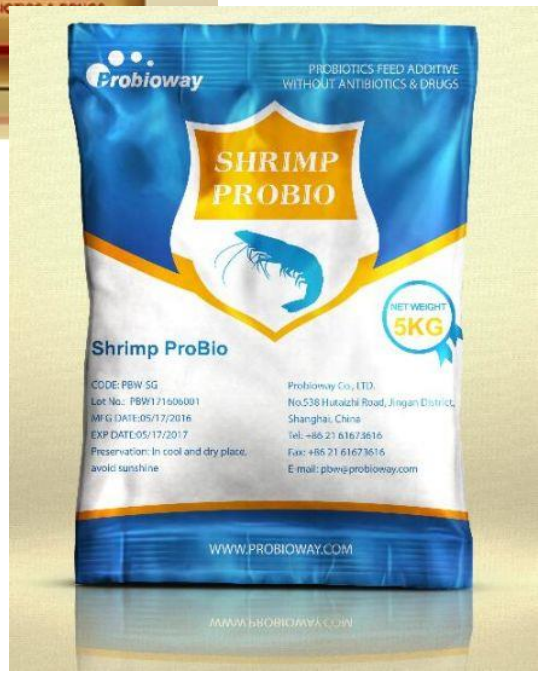
Source: BIOMIN

Source and availability of probiotics

- It may be difficult to justify the logic associated with sourcing probiotic cultures.
- The reasons for the choice of a potential probiotic often reflects the initial determination of inhibitory activity against target pathogens in vitro
- Putative probiotics have been obtained from:
  - the aquatic environment, namely water or sand
  - from fish skin mucus
  - and particularly from the digestive tract of aquatic animals





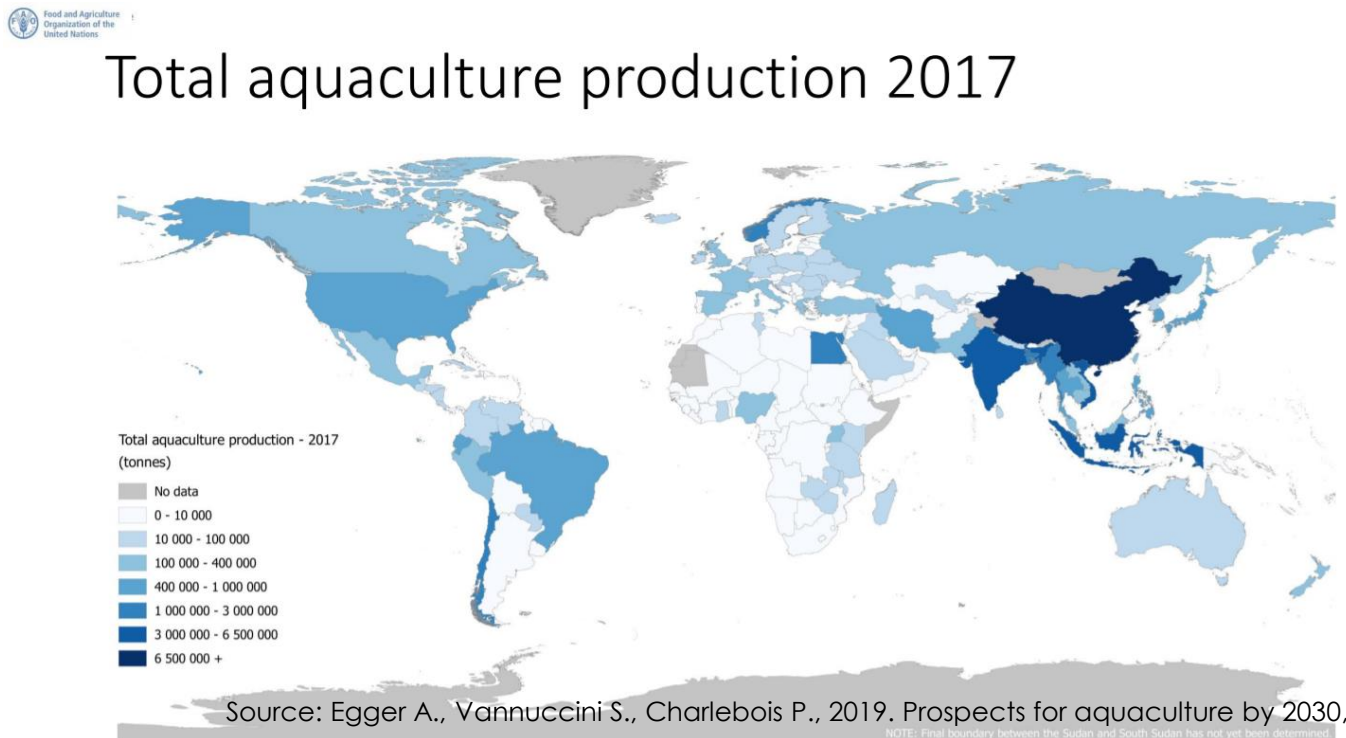


Are prebiotics the same as probiotics?

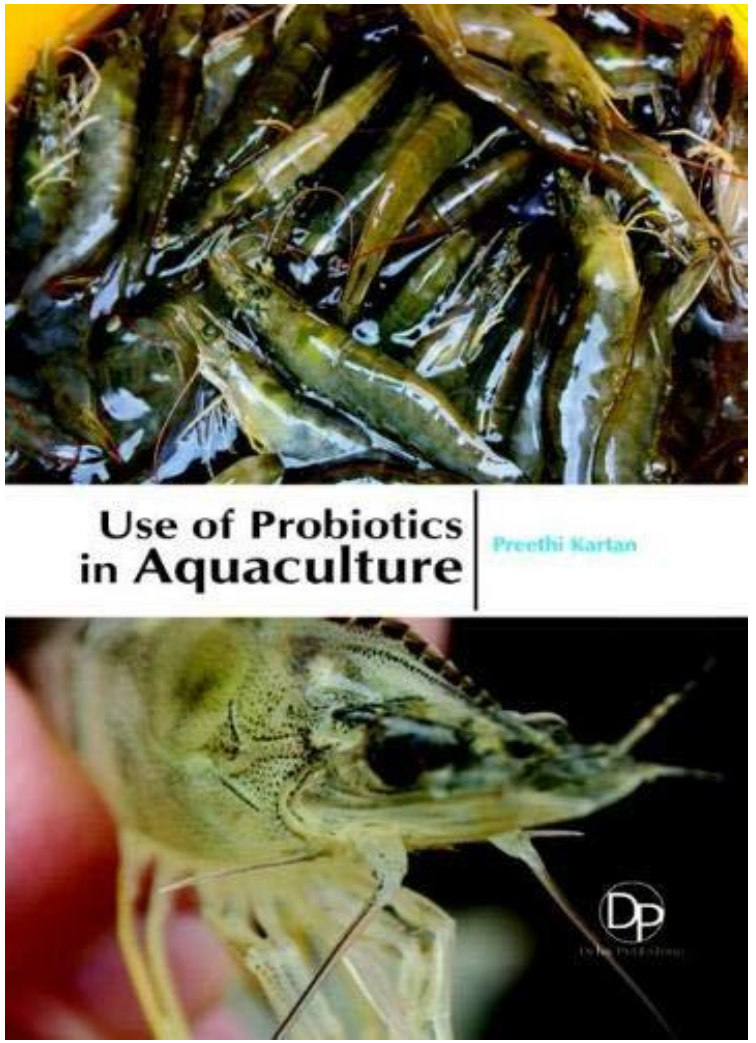
- No. Prebiotics are are nondigestible food components that selectively stimulate the growth or activity of desirable microorganisms.
    - oligosaccharides
    - proteins
    - lipids
    - peptides
    - fibers

Use of probiotics

- ***Probiotics are widely used today in aquaculture***
  - Main application field – pond aquaculture
  - RAS and biofloc technology – potential future application field
  - Most of research papers from Asia
  - Recently there is a boost in review papers

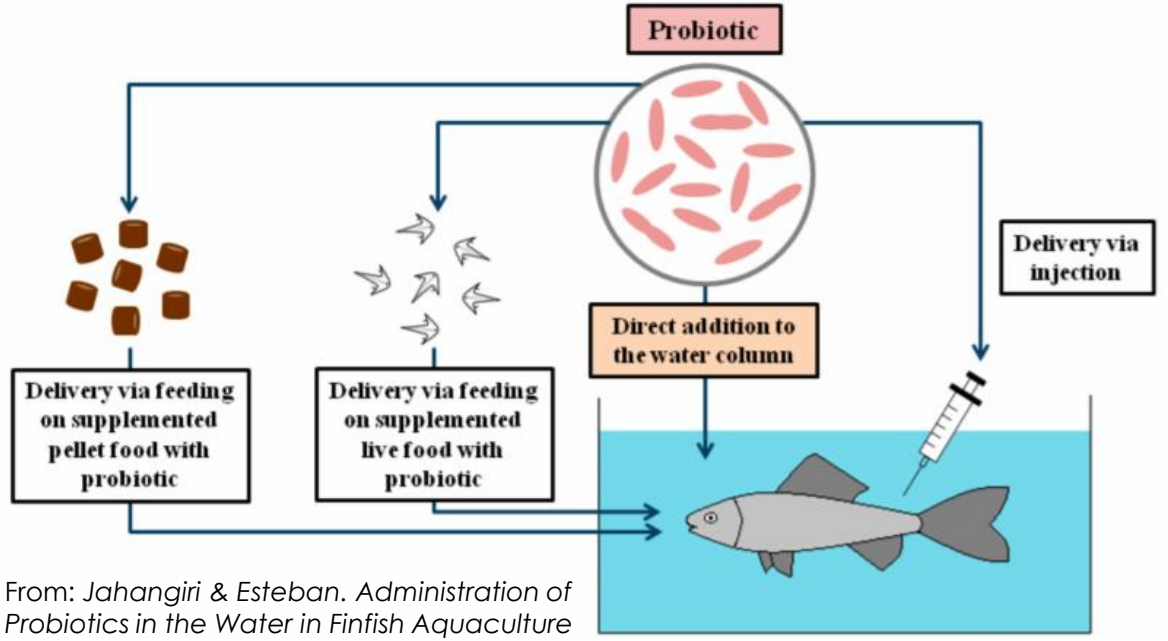


Source: Egger A., Vannuccini S., Charlebois P., 2019. Prospects for aquaculture by 2030, FAO



Use of probiotics

- **Main purposes**
  - to regenerate and improve water quality
  - to maintain microbial community of the system/eliminate pathogens
  - to improve digestive tract microflora, digestion, imunoresponse, physiological status and health of cultivated animals

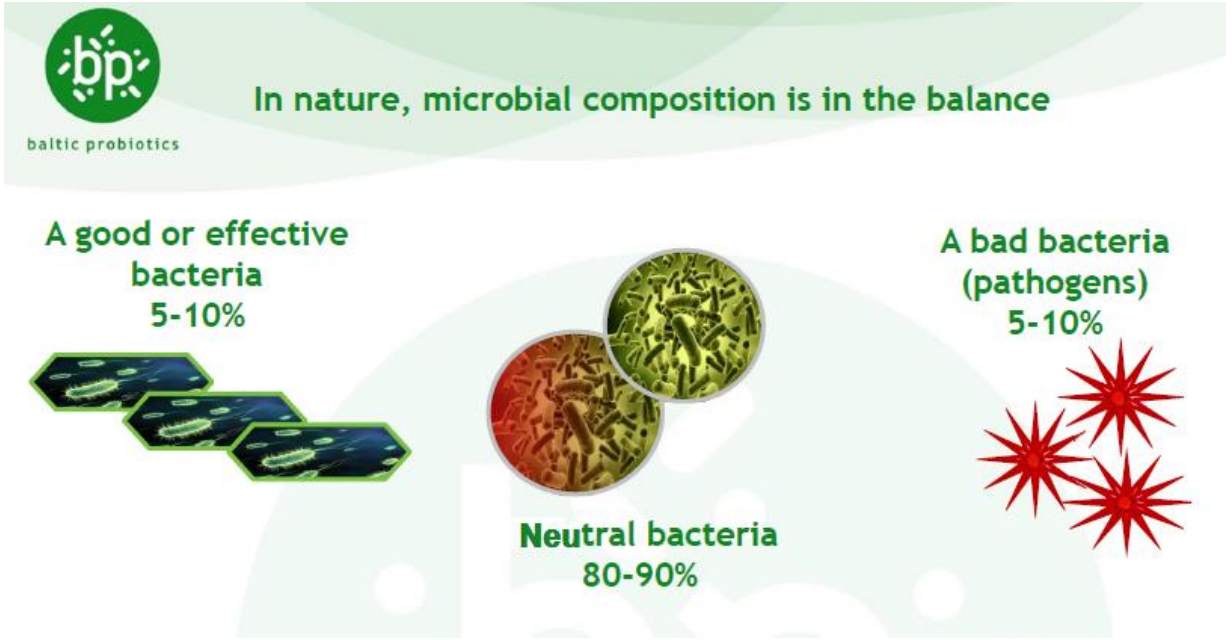


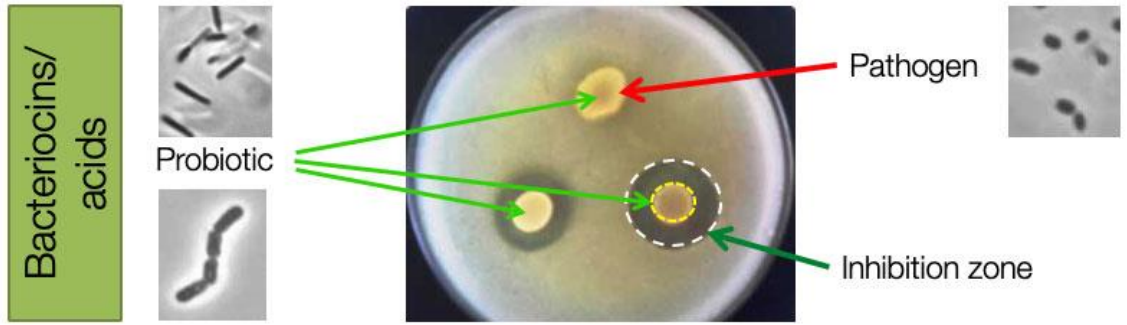
From: *Jahangiri & Esteban. Administration of Probiotics in the Water in Finfish Aquaculture Systems: A Review. Fishes 2018, 3, 33*

- **Main application methods**
  - Directly to the water
  - As feed additive
  - Inoculated life feed
  - Injections

Effect on aquaculture

- **Inhibition of Pathogens**
  - Probiotic microorganisms have the ability to release chemical substances with bactericidal or bacteriostatic effect on pathogenic bacteria.
  - The antibacterial effect is due to one or more of the following factors: production of antibiotics, bacteriocins, siderophores, enzymes (lysozymes, proteases) and/or hydrogen peroxide, as well as alteration of the intestinal pH due to the generation of organic acids.
  - Probiotic bacteria act through competitive exclusion of pathogens





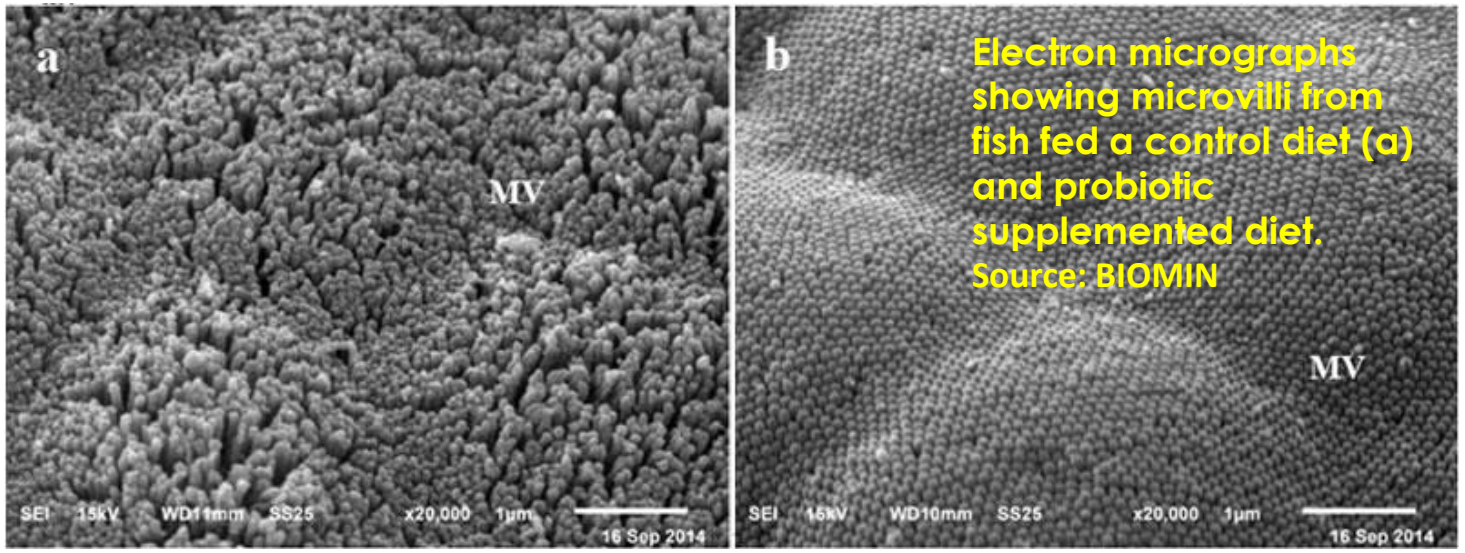
Source: BIOMIN

Effect on aquaculture

- **Immune support**
  - By attaching to different receptors, probiotics are able to interact with the host immune system.
    - to improve hematological and immunological profiles
    - can improve barrier function and modulate gene expression pathways.
  - Since aquatic animals are mostly reliant on their innate immune system, this mechanism may provide broad-spectrum disease resistance against multiple pathogenic threats.
  - Anti-inflammatory cytokines are part of a tolerance mechanism which acts to de-sensitize the host, thus it does not initiate an immune response to attack 'good' bacteria. Furthermore, they act to balance out the pro-inflammatory cytokines, thus maintaining an equilibrium within the mucosal immune system.

Effect on aquaculture

- **Improvement in Nutrient Digestion**
  - beneficial effect on the digestive processes of aquatic animals because probiotic microorganisms synthesize extracellular enzymes:
    - proteases
    - amylases
    - lipases
  - provide growth factors such as vitamins, fatty acids, and aminoacids
  - improve gut structure and function
    - LAB increase absorbative surface area of the gut, improving intestinal morphology,
      - greater villi length
      - more numerous villi
      - greater microvilli density.



Electron micrographs showing microvilli from fish fed a control diet (a) and probiotic supplemented diet. Source: BIOMIN

Effect on aquaculture

- ***Improvement in water quality.***

Baltic Blue Biotechnology Alliance project
Baltic Probiotics case

baltic probiotics

- Within the Baltic Blue Biotechnology ALLIANCE project "Baltic Probiotics" Company created 2 new recipes of probiotic-based products:

1) liquid supplement that improve and regenerate water quality;

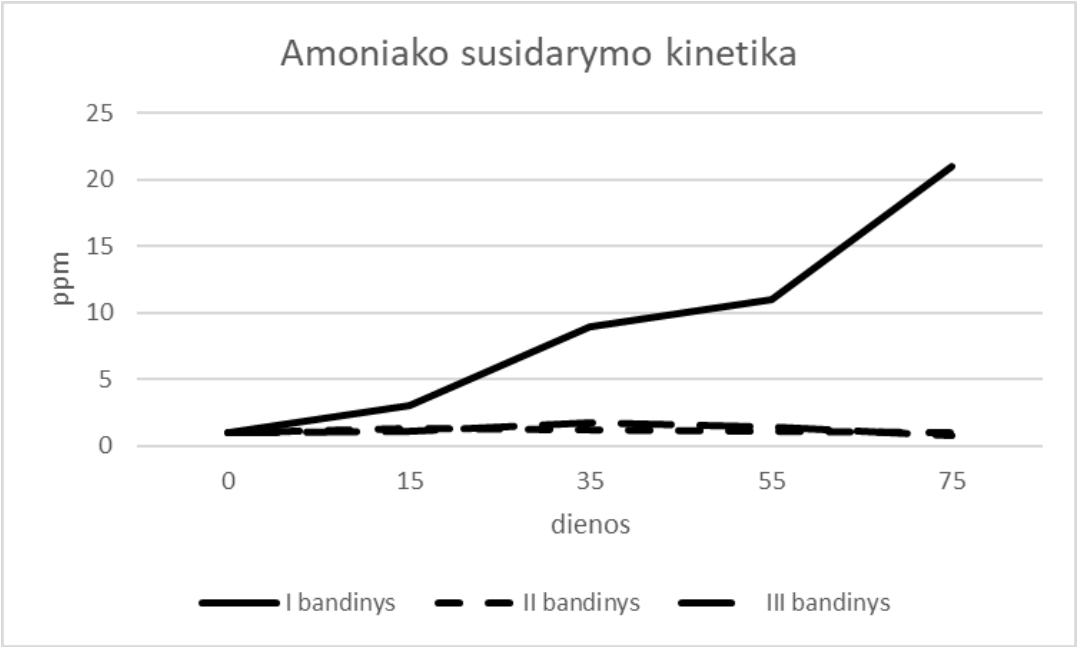
2) complementary feed additive for aquaculture fish that stabilizes the microflora of the gastrointestinal tract of fish, improves digestive processes and increases weight gain.

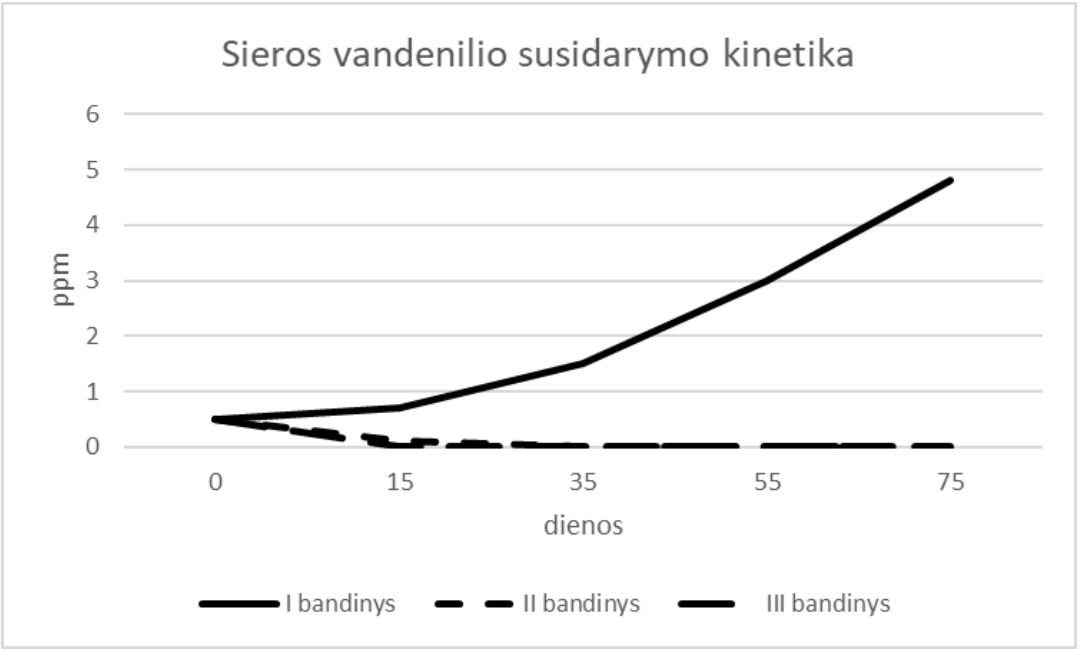
BP product impact on aquaculture water quality

I bandinys – trial with organic fertilizers (pig manure) which decompose under anaerobic contitions with natural microflora

II bandinys – trial with organic fertilizers (pig manure) which with probiotic mixture

III bandinys – trial with organic fertilizers (pig manure) with probiotcs and additional organic carbon (straw)



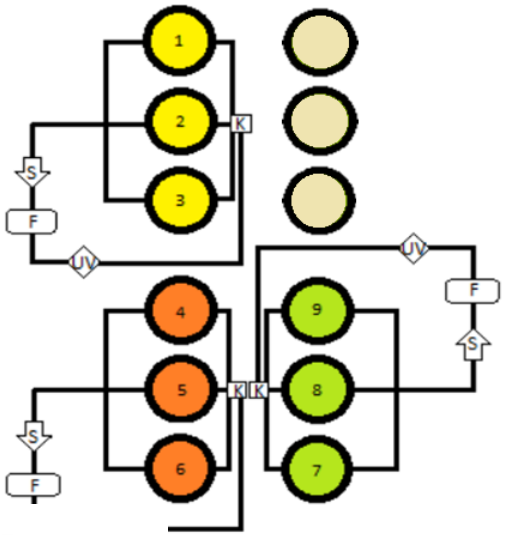


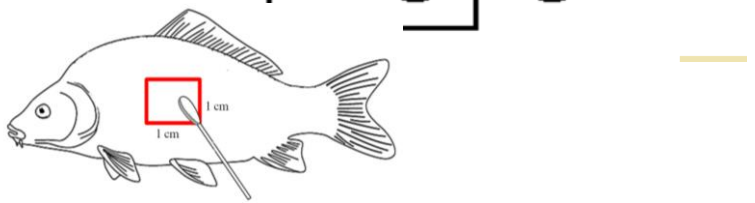
- **Decreased amonium concentration;**
- **Decreased H2S concentration;**
- **Total nitrogen didn‘t change significantly**
  - **Amonium nitrogen decreased in ~80%;**
  - **Nitrate nitrogen concentration increased ~4 times**

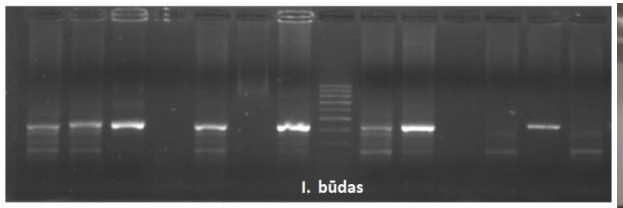
Source: „Biotechnologinių produktų naudojimas akvakultūrose“ Budrys, Balkė, AVAI

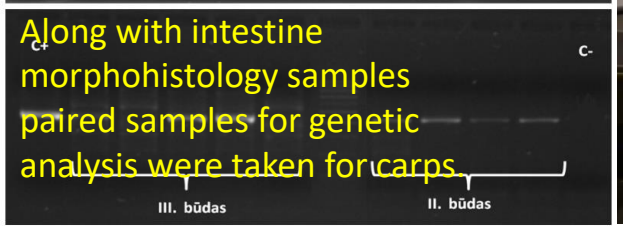
Probiotic experiment in RAS with pikeperch and carp juveniles

- 3 experimental groups:
  - Control (C) (no probiotics added)
  - Fish feed (F) (probiotics concentration of 3 ml/kg feed)
  - Water (W) (probiotics at concentration of 1 ml/m^3 water)
- The same experiment design (above) was used for pikeperch and for carp. Pikeperch experiment started with 200 individuals per tank (fry of about 1 g in weight), carp experiment – 270 fish (weighing 5 g on average) per tank. Pikeperch experiment lasted 5 weeks, carp experiment took 2 months
- Every day water main water parameters (temperature, oxygen, pH) and fish health and survival was monitored. Once every week water samples from each fish tank and biofilter were taken for nutrients (total P, NH_4, NO_2, NO_3, PO_4).
- Three replicates (before probiotic application, one month after and at the end of experiment) of fish from the tank were sampled for intestine morphology analysis.









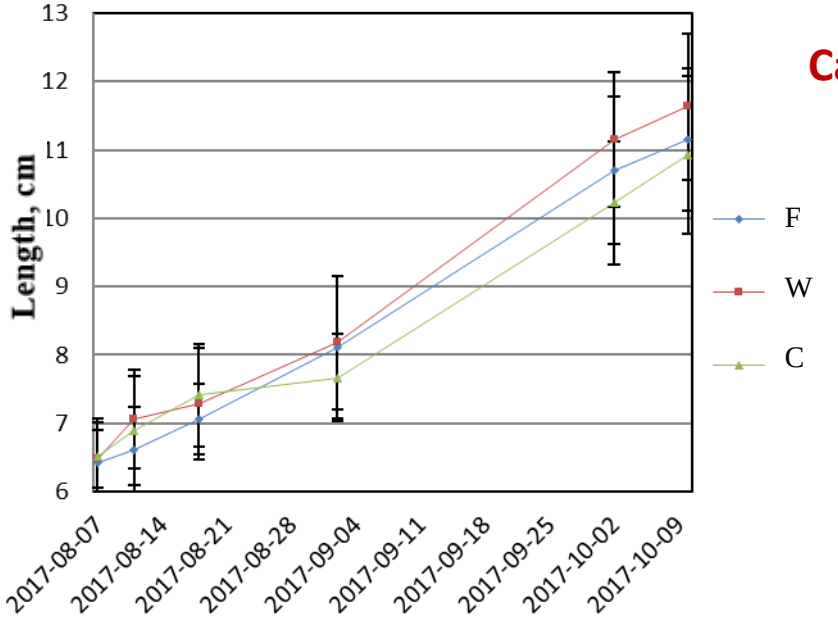
Along with intestine morphohistology samples paired samples for genetic analysis were taken for carps.

Probiotic experiment in carp aquaculture ponds

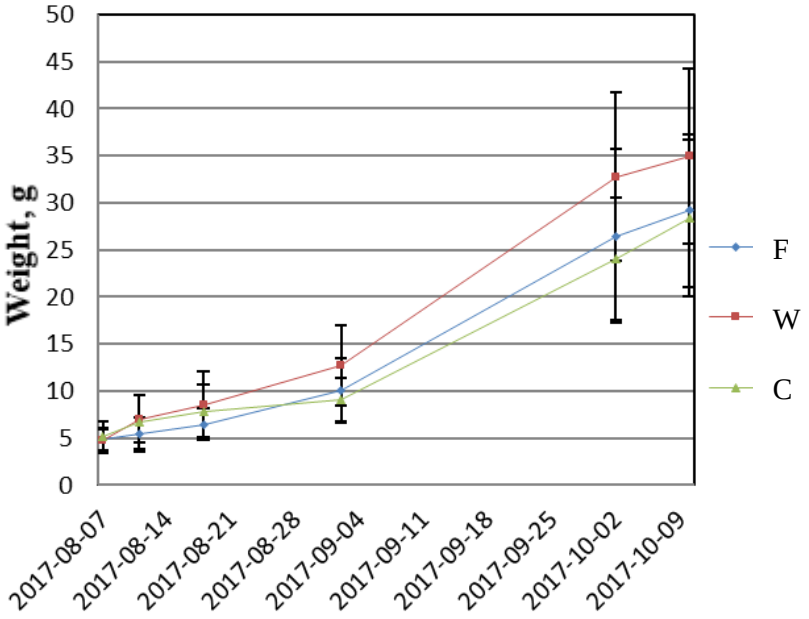
UAB KINTAI NUO 1993

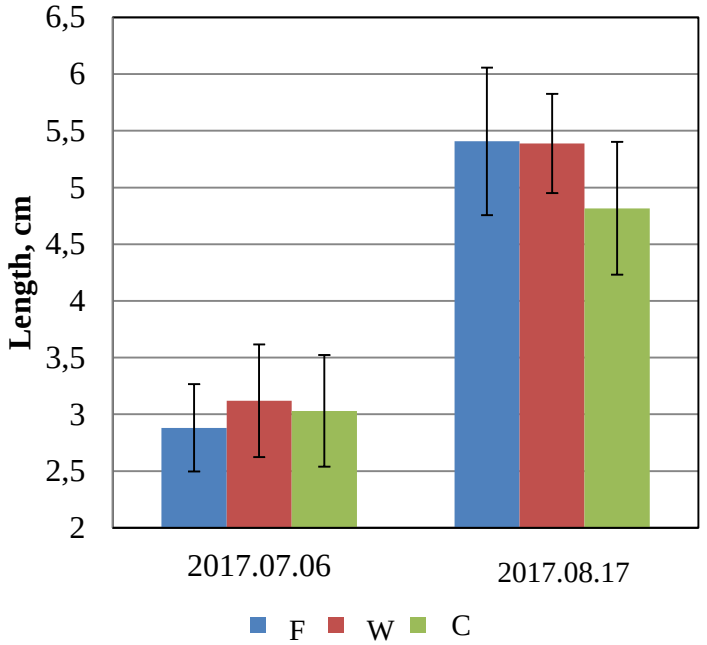
- Carp pond aquaculture experiment was performed in commercial ponds of aquaculture company “Kintai”. Two 1 ha area ponds of first summer fish were available for experiment (no replicates). The task was to assess ecosystem response to probiotic treatment by measuring water chemistry, biogeochemical nutrient cycling, primary production, zooplankton and fish growth.
- Experiment took from June to July.
- Surface water samples were analysed for nutrients (total N, total P, NH_4, NO_2, NO_3, PO_4) and bacteria abundance. Water temperature and oxygen concentration was tracked daily using automatic data loggers.
- Primary production evaluated as *chl a* concentration, zooplankton density was counted and fish growth was measured (length and weight) before probiotic treatment (June 6), 2 weeks and 4 weeks after the treatment of one (Probio) pond.

Fish juvenile growth in RAS

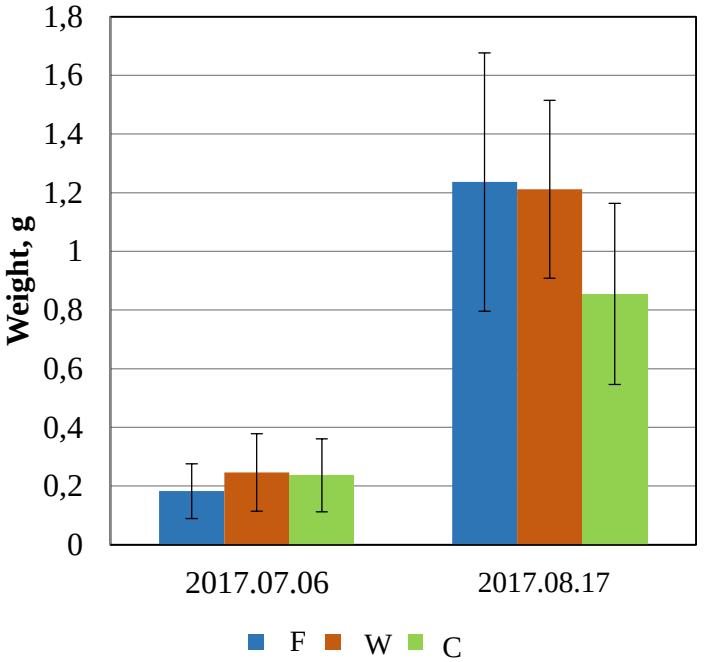


Carp

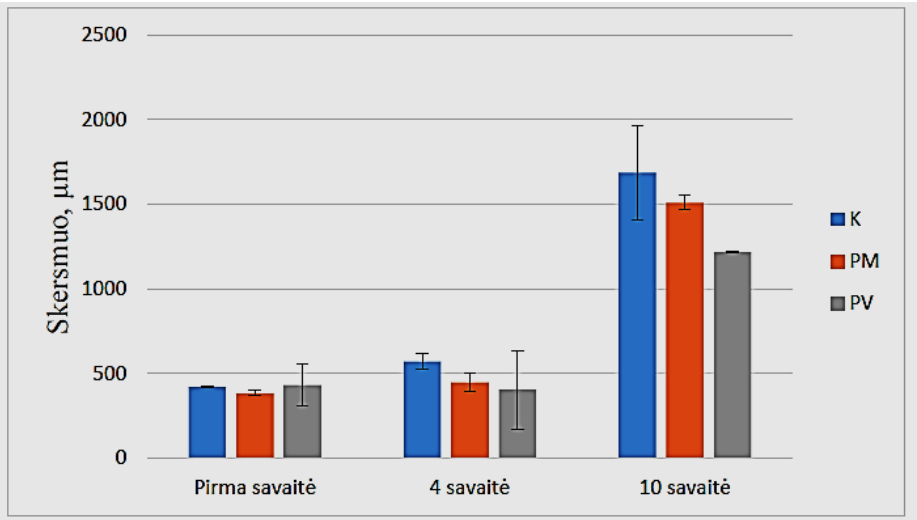


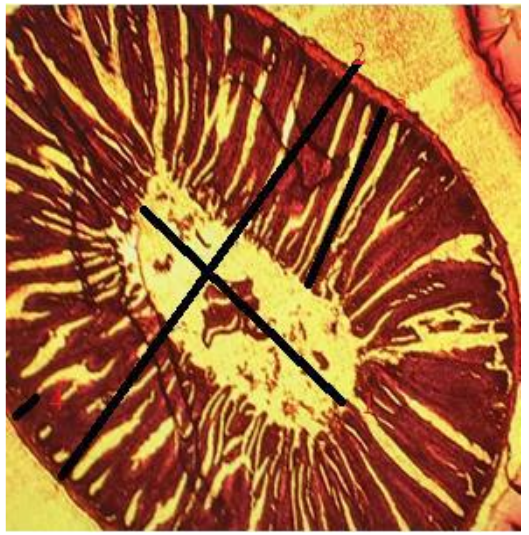


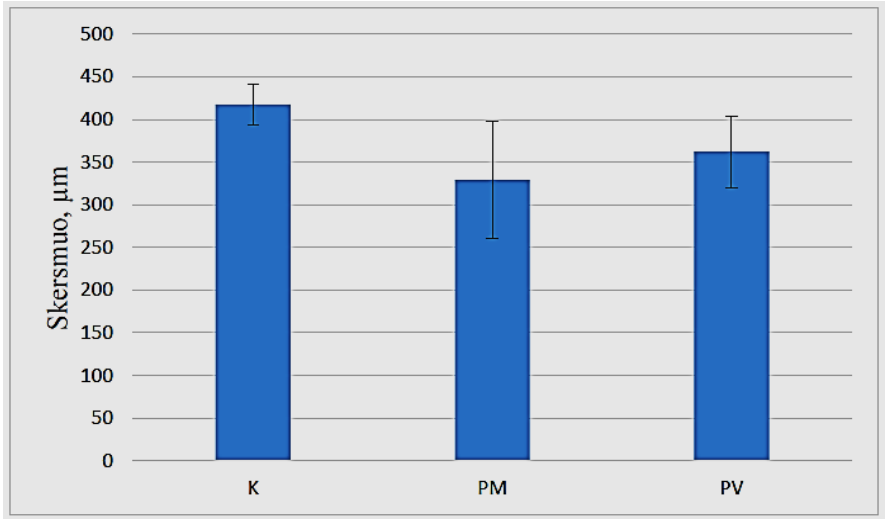
Pikeperch

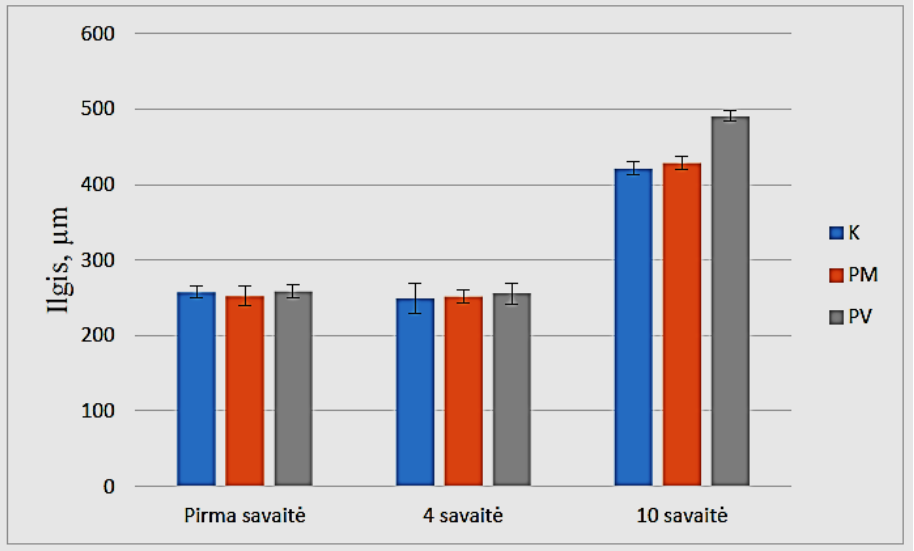


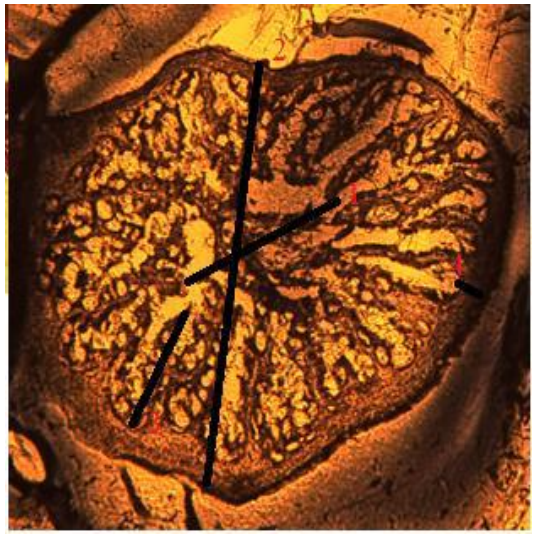
Probioc effect on fish juvenile gut morphology

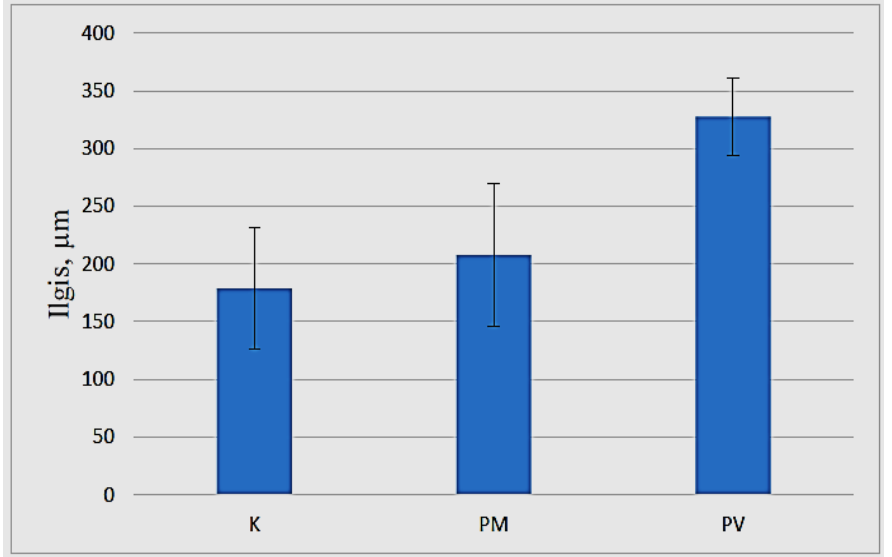




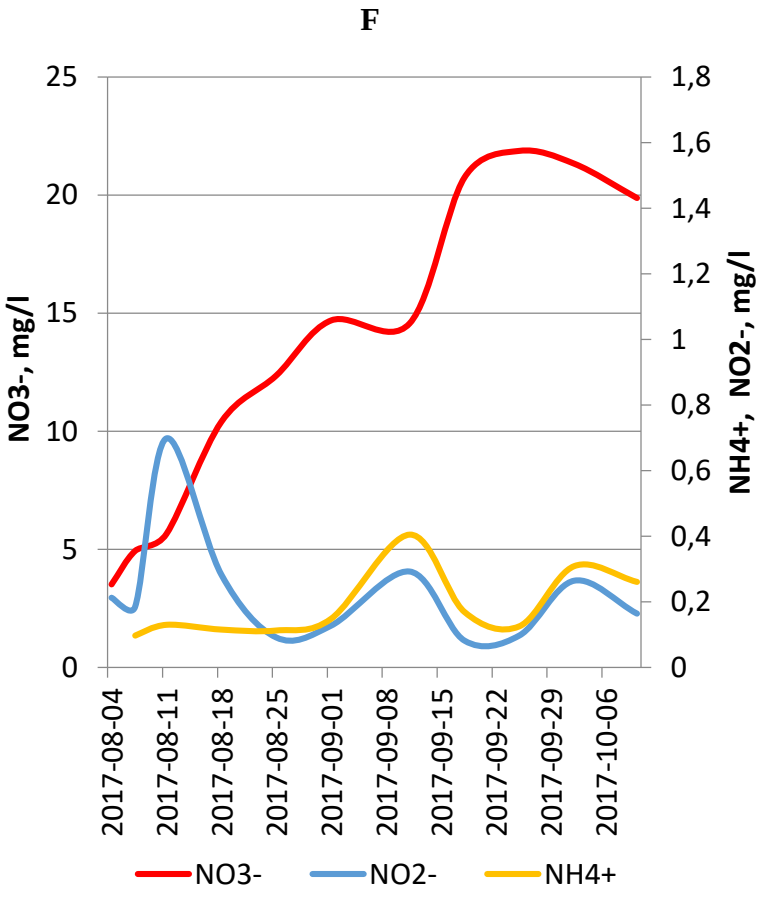


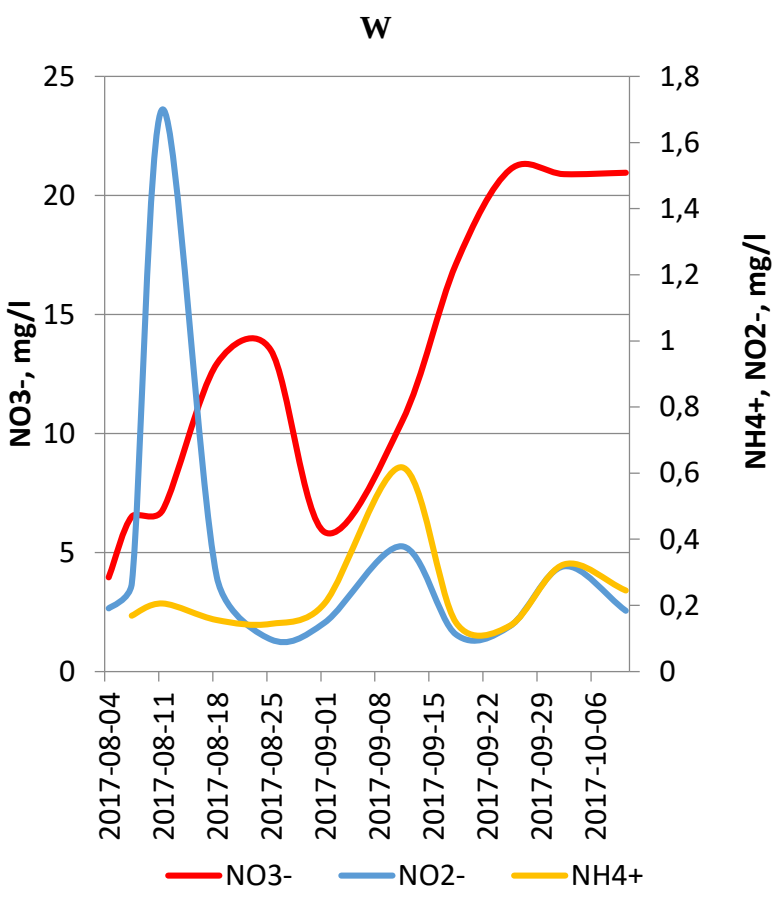


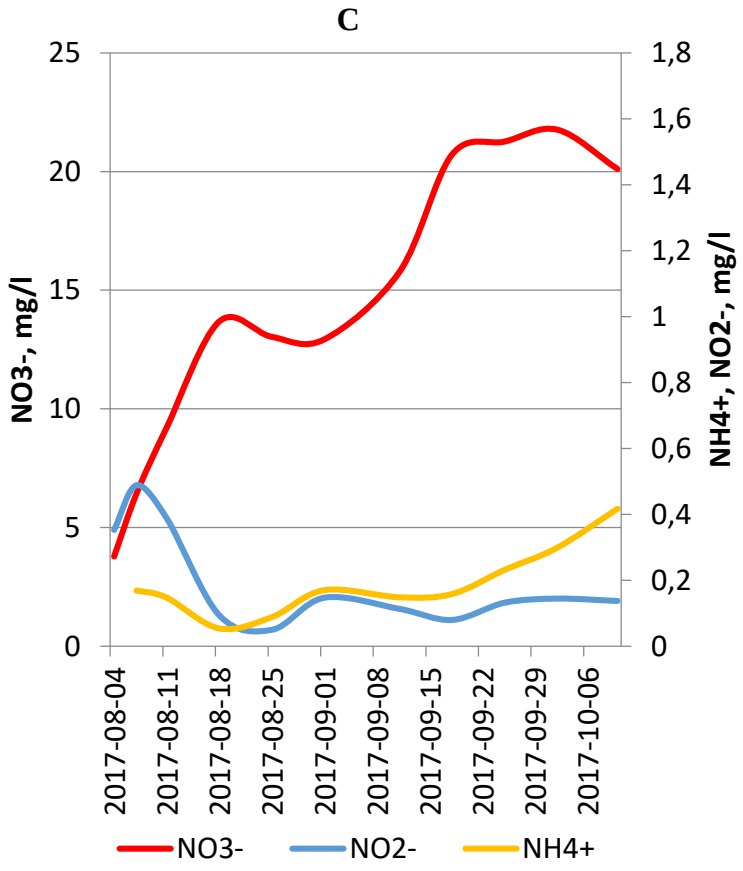




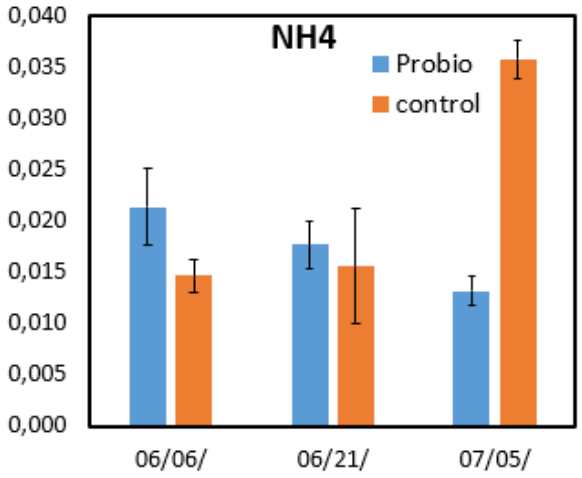
Probiotic effect on RAS water quality

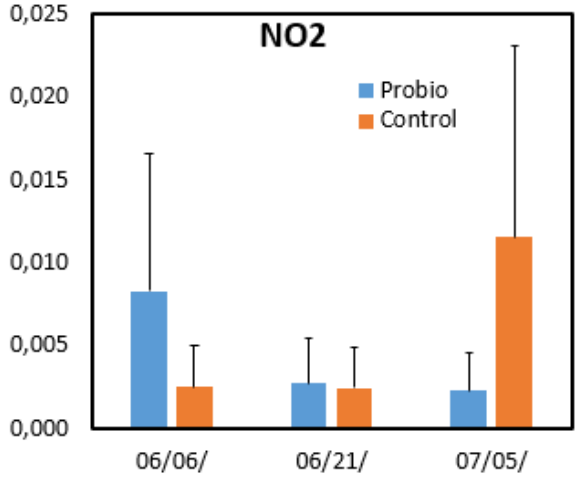


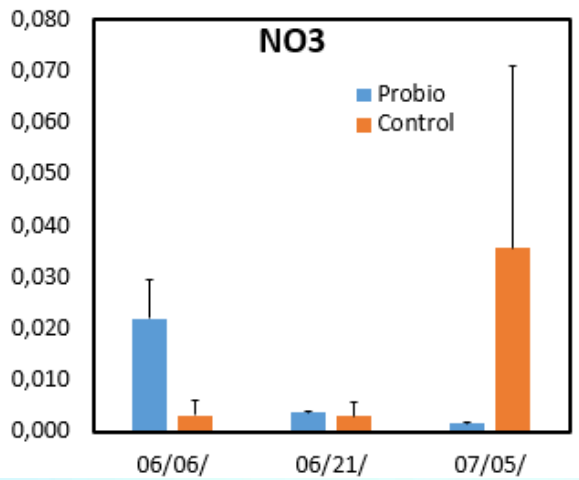


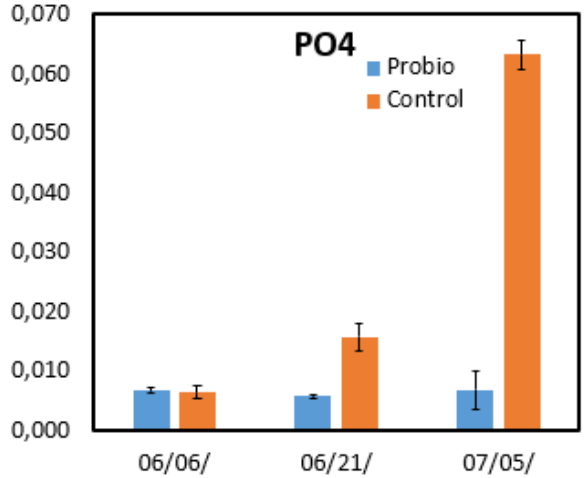


Probiotic effect on water quality in carp aquaculture ponds



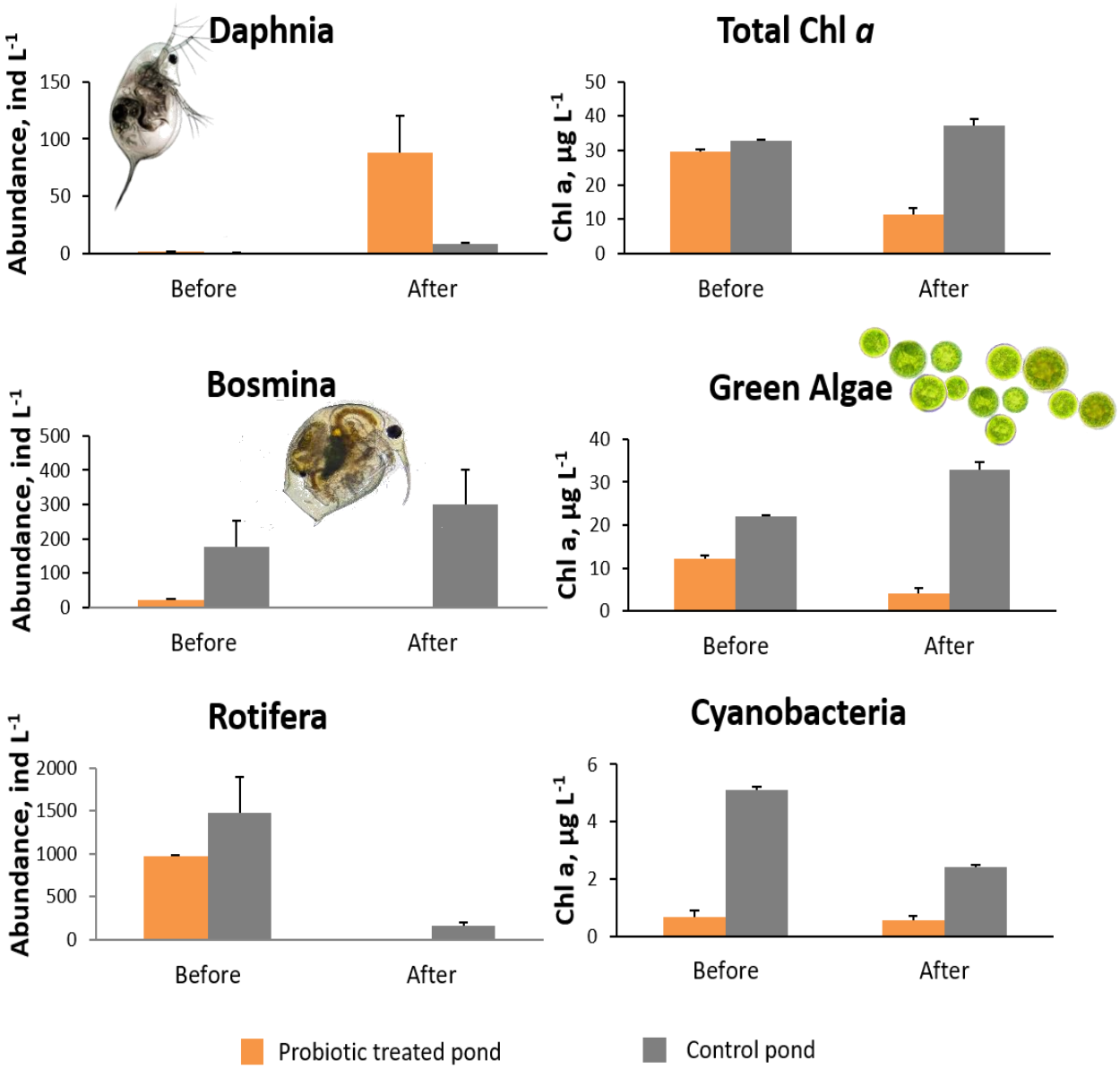




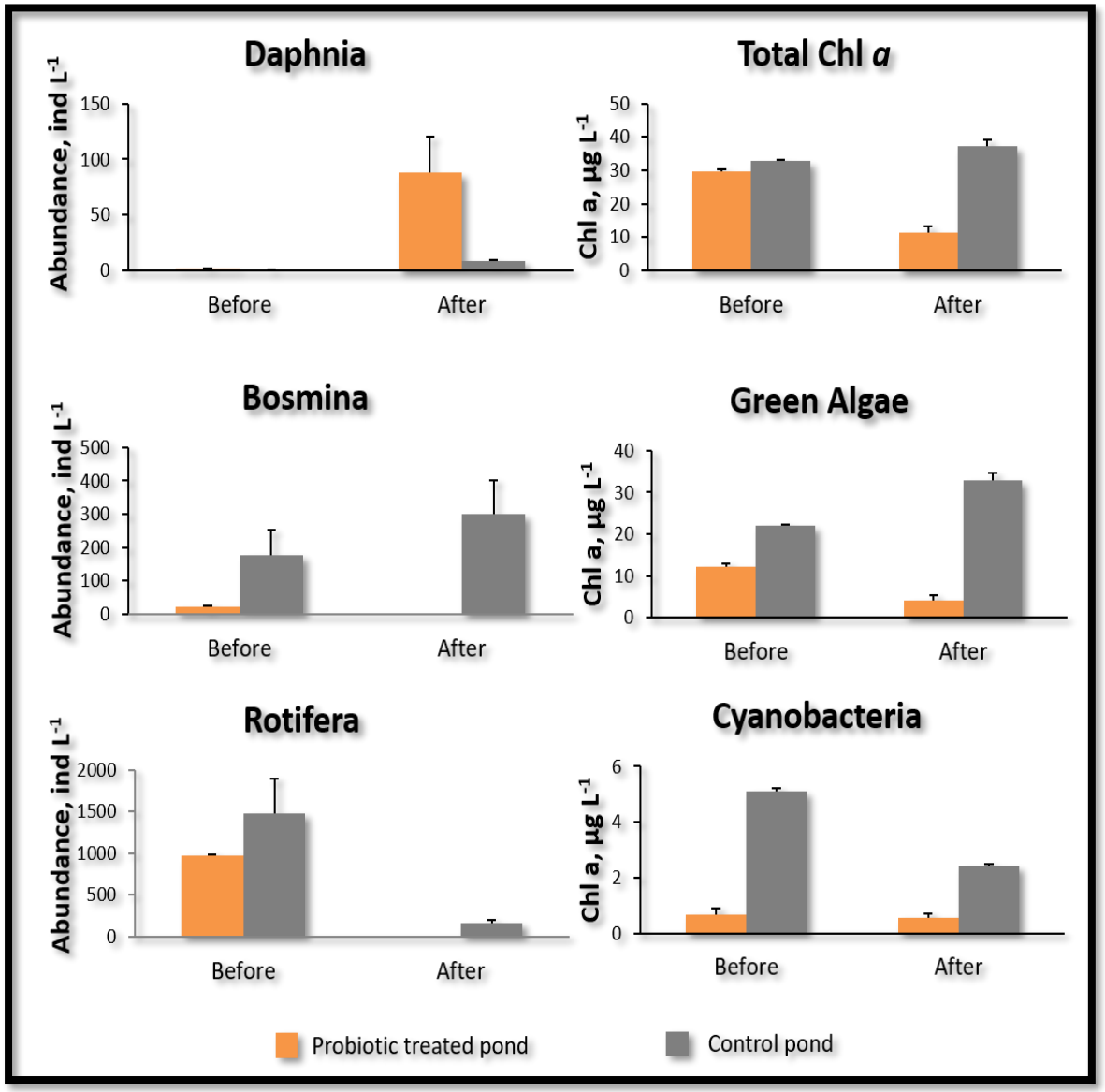


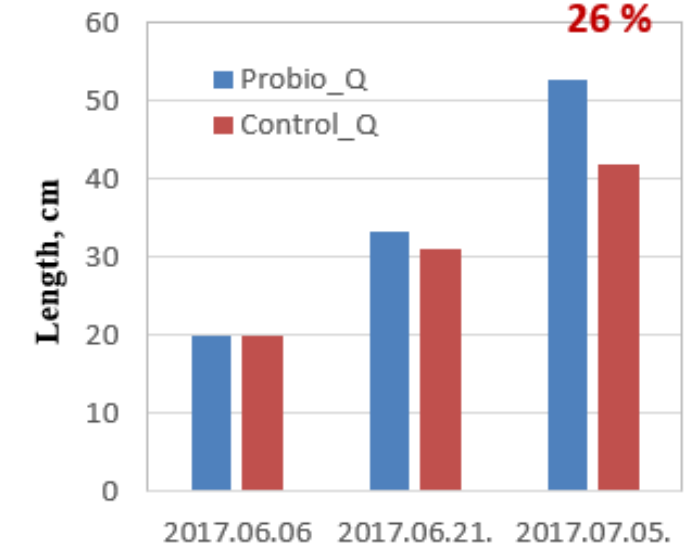
Ecosystem impact

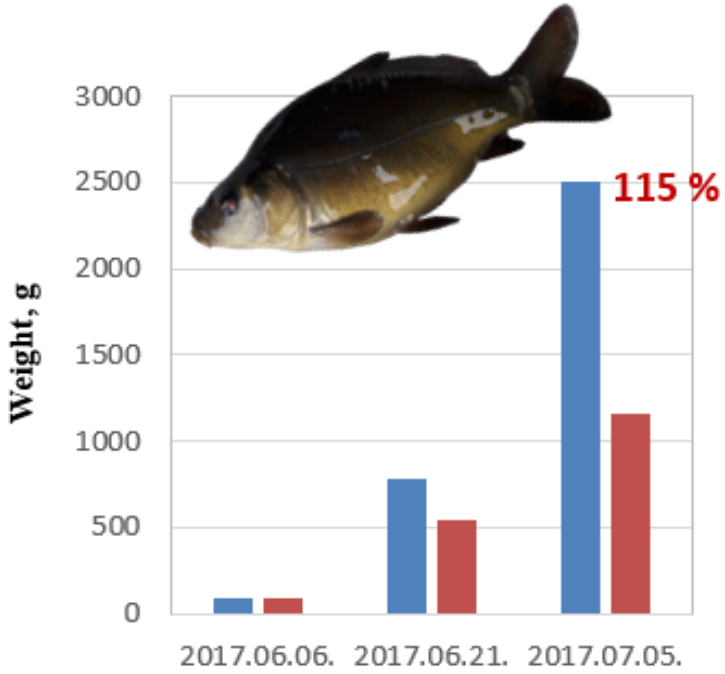
- After two week water was significantly clearer in probiotic-treated pond (0.7 and 0.3 m respectively).
- In one month *Daphnia* densyty increased ~3.4 times in experimental pond comparing to untreated pond.
- Intensive *Daphnia* growth led to higher phytoplankton grazing rate – 2.6 times drop in Chl a concentracion. Control pond had no significant decrase in phytoplankotn biomass.



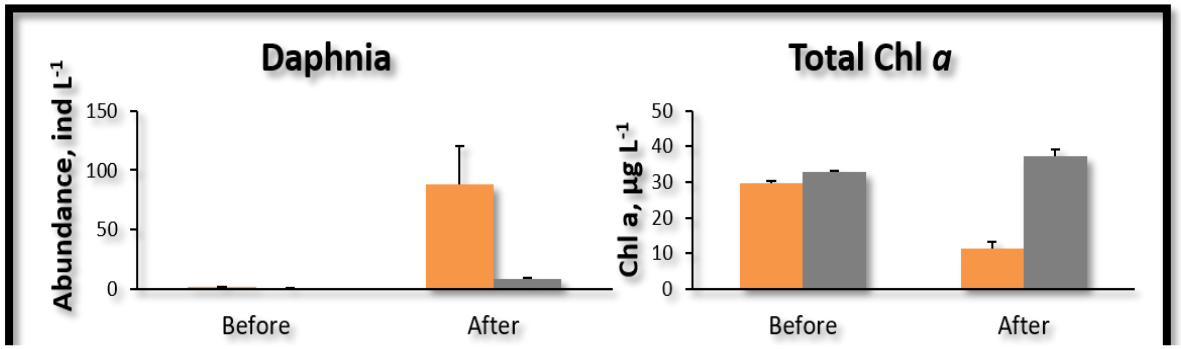
Ecosystem impact on carp growth

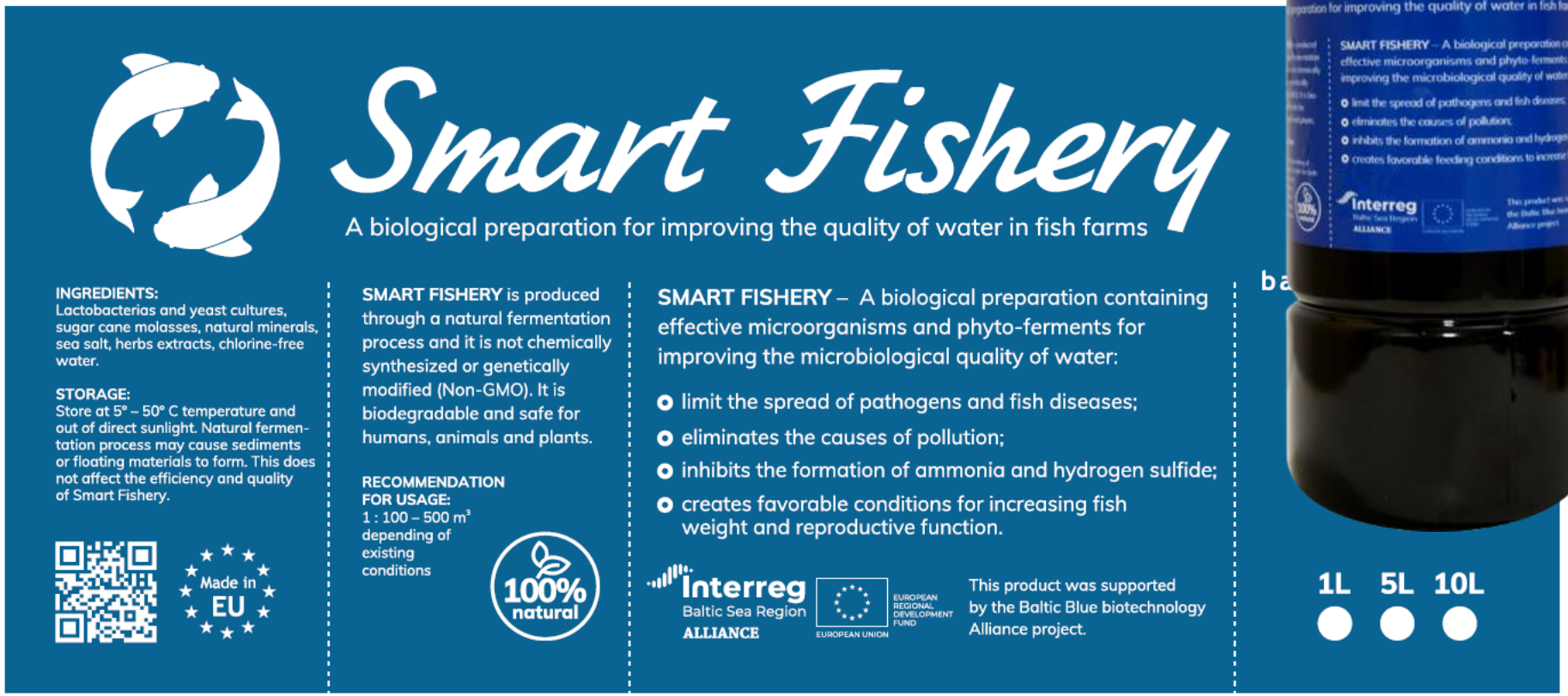


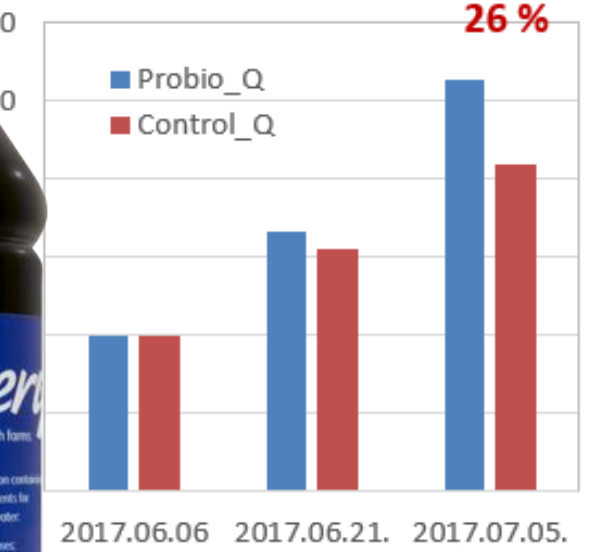


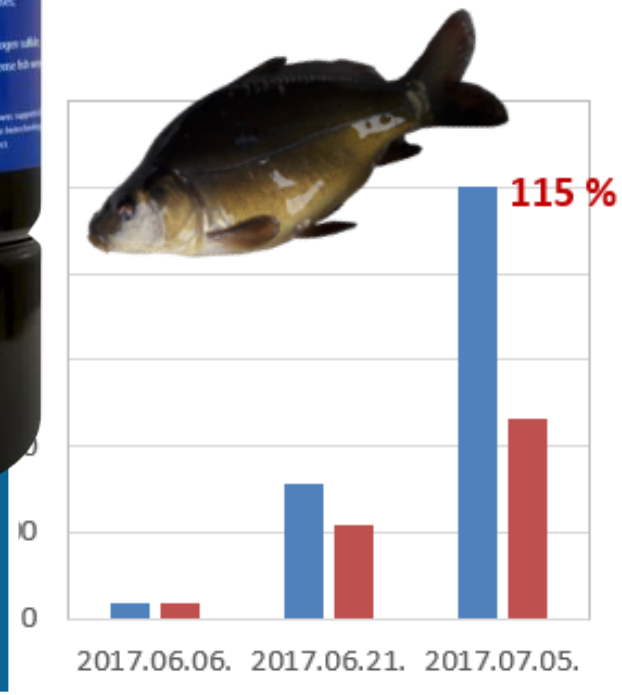


Ecosystem impact on carp growth









Thank you!

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