Vaccination of sea bass against Viral Nervous Necrosis (VNN) and characterization of protective immunity

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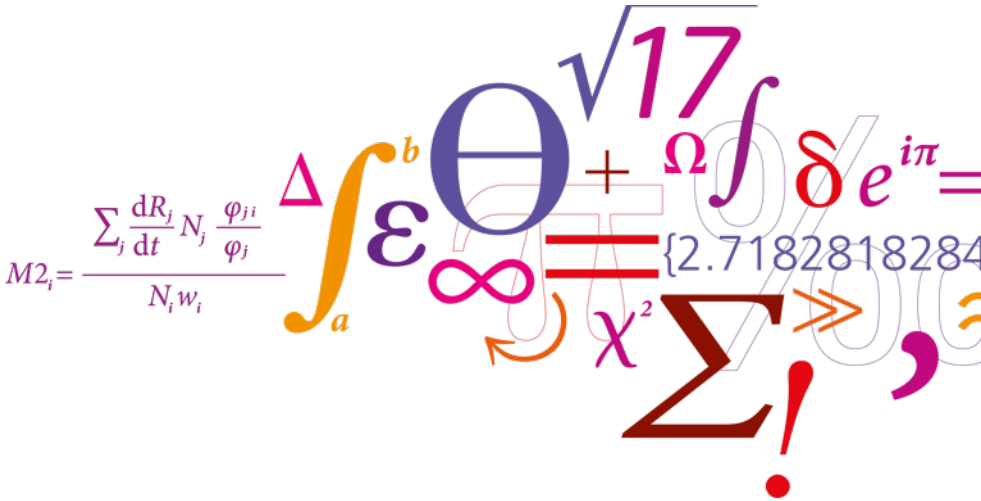


DTU Aqua
Institut for Akvatiske Ressourcer

Agenda

Nodavirus
- Disease
- Virus

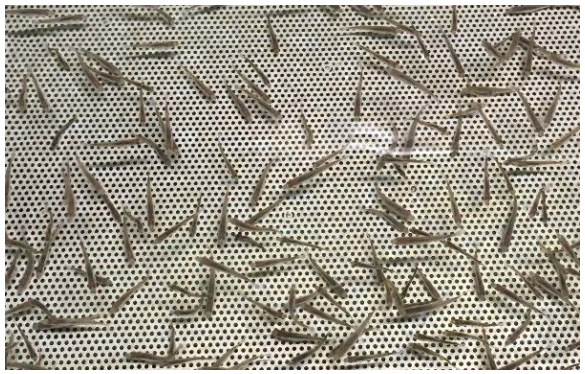
Aim of phd project

Virus like particles

Trials (initial)

Partners

Viral encephalo- and retinopathy / Viral Nervous Necrosis



- Causes high mortalities in young fish (fry)
- Older fish get chronically infected and can be asymptomatic carriers (histopathologic lesions present)
- > 40 marine species affected (also found in some fresh water species), including sea bass and recently also sea bream.

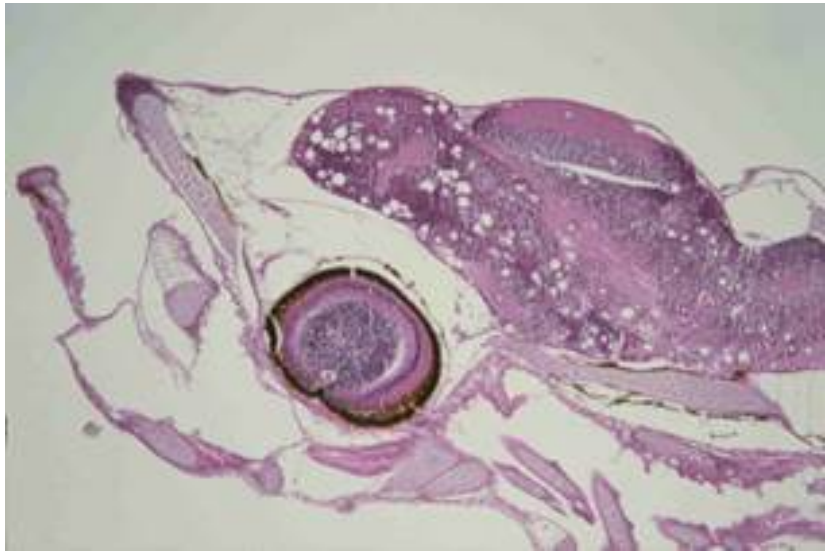
Low et al 2017

Viral encephalo- and retinop / Viral Nervous Necrosis

Sea bass with VNN; abnormal swimming pattern. (Video: Panos Christifilogiannis, www.vimeo.com)

Clinical signs: abnormal sprial swimming behaviour, darkening of skin pigmentation, loss of buoyancy control and lethargy

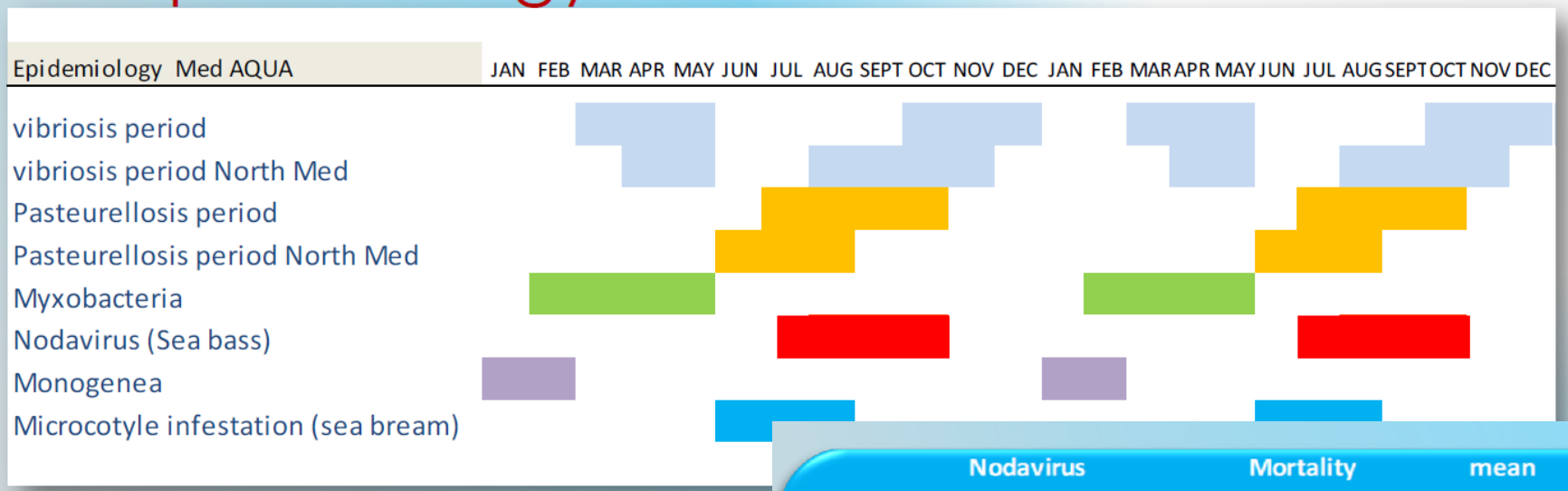
Sea bass with VNN; abnormal swimming pattern and death (Photo: Niccoló Vendramin)



Histopathologic lesions caused by NNV (Photo: Niccoló Vendramin)

Viral encephalo- and retinopathy / Viral Nervous Necrosis

Epidemiology on the fish farm



	Nodavirus Prevelance first summer	Mortality in the first summer	mean weight first summer	Nodavirus Prevelance second summer	Mortality in the second summer	mean weight first summer
Greece[1]	70%	25-30%	2-25g	30%	10-20%	300g
Spain[2]	100%	10-20%*	20-60g	100%	5-10%	300g

* SPAIN :Mortalities can reach 50-60% at any stage if secondary infections are present (Vibriosis / Pasteurelosis) due to Lack of vaccination strategies

1 Source: Dr. Nancy Dourala

2 Source: Dr. Carlos Zarza

Kilde: Aquark 2013

Viral encephalo- and retinopathy / Viral Nervous Necrosis

Great economical losses in mediteranean aquaculture

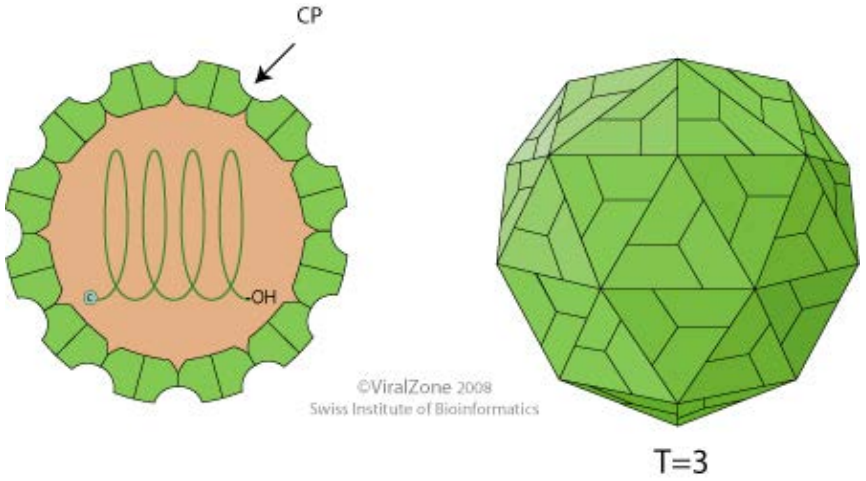
- High mortalities – production losses
- High costs in terms of cleaning and disenfection after an outbreak
- No cure
- No prophylactic measures
  - (New inactivated vaccine just got launched in Spain, Italy, Croatia and Greece by Pharmaq)

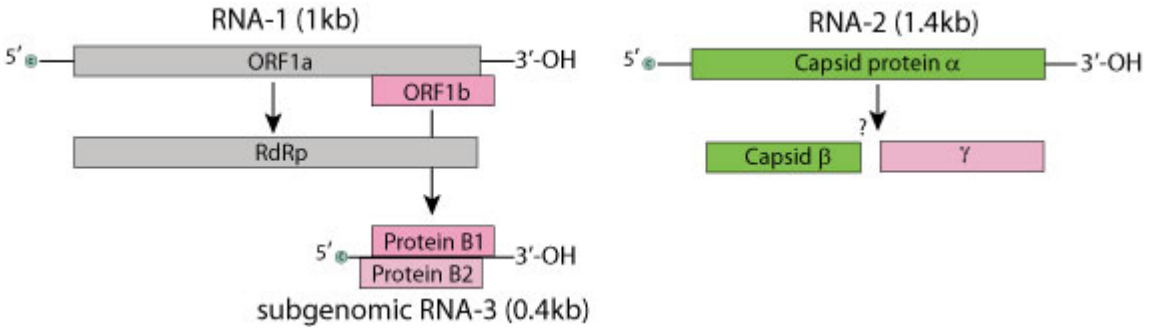
Difficult to avoid

- Spill-over from wild fish
- Vertical transmission
- Can be spread by vectors (boats, equipment etc.)
- Relatively resistant (resistant to acid conditions and up to 37ºC)

(OIE Aquatic manual (Chapter 2.3.12.))

Betanodavirus – Nervous Necrosis Virus (NNV)





Source: https://viralzone.expasy.org/47?outline=all_by_species

Single stranded positive sense non-enveloped RNA virus. Icosahedral capsid (T=3) ranging from 29 to 35 nm in diameter.

RNA 1 (3.1 kb) encodes polymerase and subgenomic RNA 3

RNA 2 encodes the coat proteins (CP)

Causes VER (Viral encephalo- and retinopathy), also called VNN (Viral Nervous Necrosis) in several species

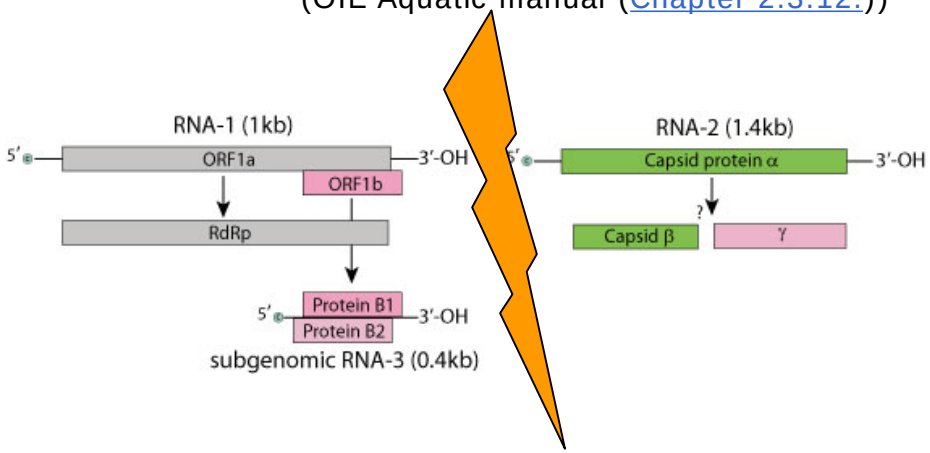
NNV strains

Genotype	Sero-type	Target host fish	Optimum growth temperature
SJNNV	A	Striped Jack	20-25ºC
TPNNV	B	Tiger Puffer	20ºC
BFNNV	C	Cold-water fish (atl. halibut, atl. cod, flounders etc.)	15-20ºC
RGNNV	C	Sea bass, groupers etc.	25-30ºC

(OIE Aquatic manual (Chapter 2.3.12.))

Host specifity (and genotype) is determined by the variable T4 region of the RNA2 (Nishizawa et al 1997)

Recently **SJ/RG** and **RG/SJ** reassortant virus has been isolated from outbreaks at mediteranean fish farms with sea bass and sea bream (Panzarin et al 2012)

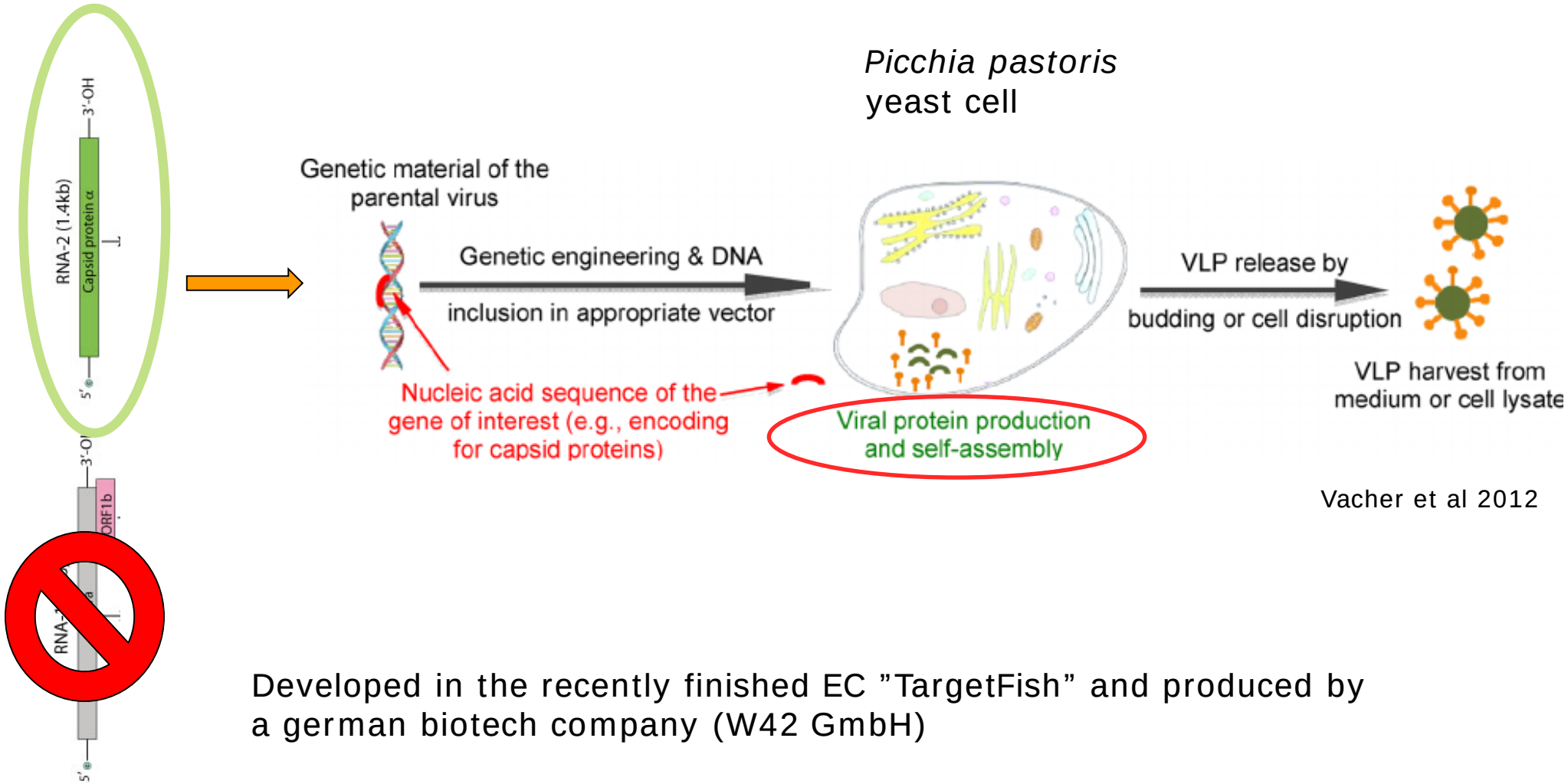


(OIE Aquatic manual (Chapter 2.3.12.) and Toffolo et al 2007)

Aim of phd project

- Optimization of a vaccine prototype against nodavirus infection based on recombinant VLP's (Virus Like Particles)
- Characterization of the vaccine induced immune response, protective mechanisms and safety aspects
- Testing of different vaccination strategies, including dose, delivery and adjuvant aspects under laboratory conditions
- Assesment of protection across viral genotype/serotype under experimental conditions
- Participate in vaccine testing under field conditions

Virus like particle (VLP)



Picchia pastoris
yeast cell

Vacher et al 2012

Developed in the recently finished EC ”TargetFish” and produced by a german biotech company (W42 GmbH)

NNV-VLP advantages

- All surface antigens from the pathogen
- Non-infectious
- Immunogen size (same size as the virus)
- Easy to produce in high yields (pichia platform)

Trials

Trials – susceptibility of different families

Eastern Mediterranean

Western Mediterranean

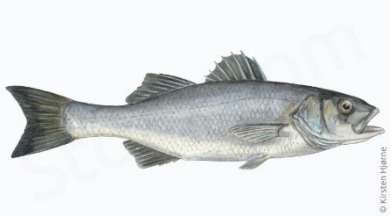
Infection with

- RGNNV
- RG/SJNNV

By IM injection (10^5)

4 weeks

Survival rate?



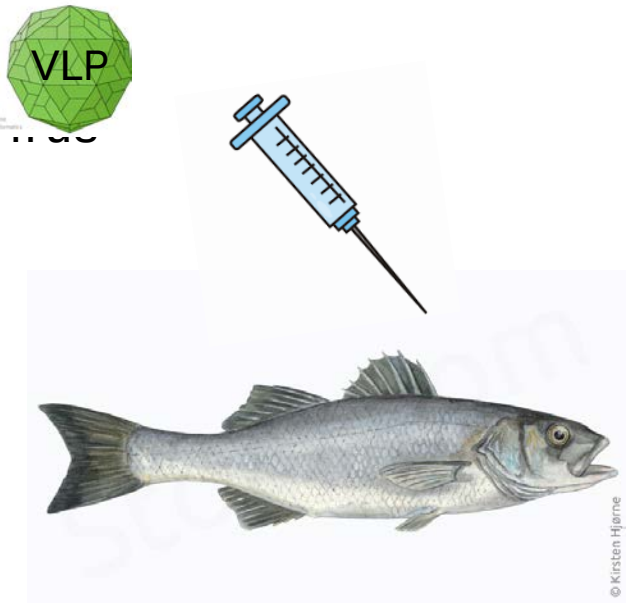
Atlantic

Aim for infection model:

60% disease/euthanization in naïve fish

Trials - vaccination

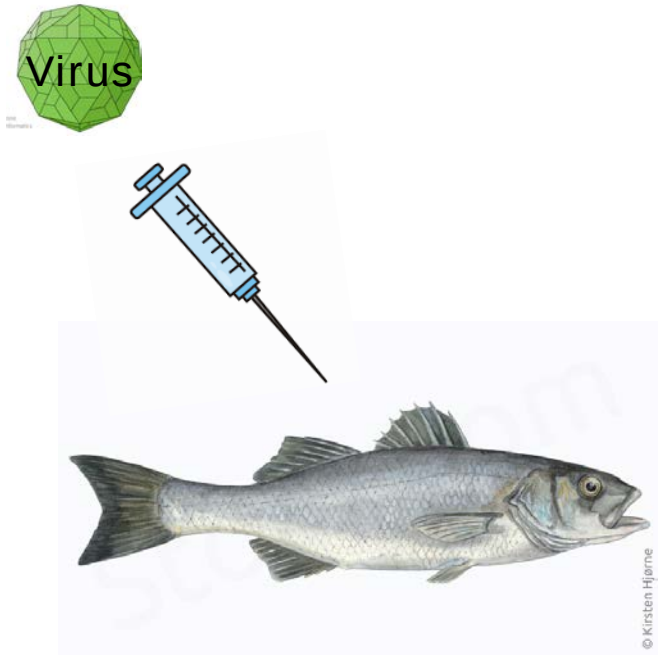
Vaccination



- V...

- Dose: ?
- Route: injection, maybe later dip immersion of larvae.
- Age: fingerlings
- Adjuvant: oil, commercial adjuvant

Challenge



- Mortality
- Detection of neutralizing antibodies
- Detection of virus in sick/euthanized
- Gene-expression

External partners

W42 GmbH

- German biotech company, who produce the VLP in pichia pastoris

Horizon 2020 - EU financed project