Aspects of experimental and field trials with fish

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Irish Medicines Board

Assessor Training – Fish Vaccines

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Background for this talk: clinical trial experience at VESO Vikan

- VESO is a limited company (indirectly) controlled by the Norwegian Government (51% Ministry of Food and Agriculture, 49% SIVA – the Industry Development Corporation of Norway)
- VESO is operating under commercial conditions without any basic financial support

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Main activities of VESO

- **Aquamedical CRO - VESO Vikan in Namsos, Mid-Norway**
- **Expert reports (efficacy, safety of fish medicines and vaccines)**
- Expert consulting in aquaculture health management and animal welfare
- Health management operations of wild fish stocks (hatceries, gene banks)
- Distribution of animal vaccines in Norway (“national pharmacy”) for veterinary vaccines including fish vaccines

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VESO Vikan experimental facility

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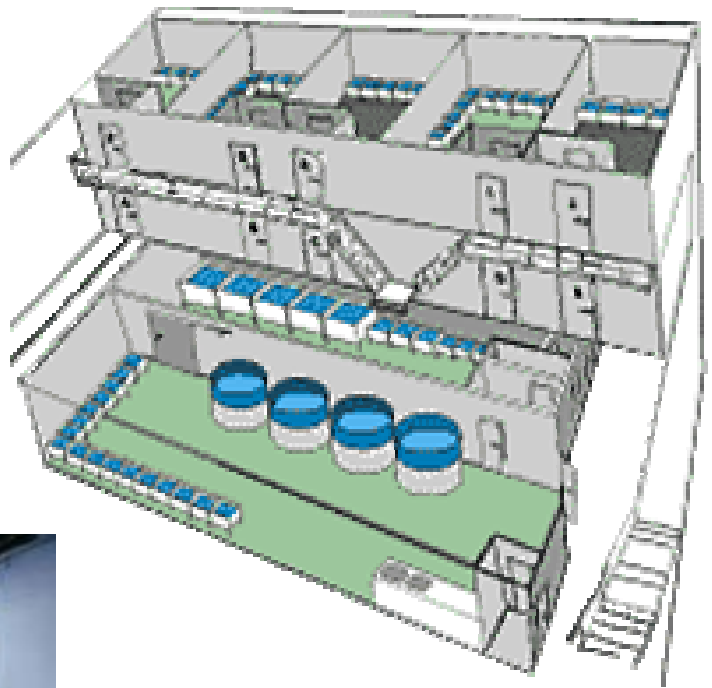
Large-scale wet laboratory for biomedical trials in fish

Unique capacity for tank experiments:

- 200 tanks 180-15000 l
- 450 mini-tanks 8 l

➢ 50 000 tank-days/year



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Diversity of technical capacity

Can hold any fish size and species available at any salinity and water temperature

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Extensive trials experience

Unique trials experience over 17 years of operation:

> 1700 assignments/contracts until today

- Diverse experimental models (bacteria, viruses, parasites, safety) available in salmonids but also in sea bass, flatfish and cod

GLP accredited since 1996

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VESO Vikan hatchery

Supply of SPF experimental salmonids

Field trial capacity ca. 250 000 smolts

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VESO Vikan marine field unit

Commercial scale marine A. salmon site

4 marine net pens – ca. 200 000 fish

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Important aspects when conducting (or assessing) fish trials

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Fish are not homeotherms!

Physiological processes in fish are principally temperature-dependent

- Example: embryonic development
  - A. salmon: ca. 500 degree-days to hatching
  - R. trout: ca. 350 degree-days to hatching
- Growth (% daily weight gain)

Comparison of treatment groups is therefore only meaningful when held under the same water temperature, or over the same (seasonal) period of time

Temperature-dependence of medicinal drug metabolism (1)

OTC single-dose study in R. trout	**16°C**	**10°C**	**5°C**
Max serum concentration	1 h	12 h	24 h
Half-life in serum	4.8 days	6.1 days	8.9 days

Björklund and Bylund, Aquaculture 84, 363-372 (1990)

Temperature-dependence of medicinal drug metabolism (2)

Flumequin single-dose study in R. trout	**3°C**	**13°C**
Elimination half-life (intravascular dose)	569 h	137 h
Clearance rate (intravascular dose)	0.005 l/kg*h	0,018 l/kg*h
Max plasma concentration (oral dose)	1.14 µg/ml	1.93 µg/ml

Sohlberg et al. Aquaculture 119, 1-10 (1994)

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Temperature-dependence of immune responses

- First reported by Cushing 1942 (!)
- Firmly established in carp and salmonid species in the 1970-80ies

Limited value of serosurveillance of fish sampled from cold waters (<10°C)

In coldwater fish, antibody response assays tend to yield outcomes near their ”limit of quantification”

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Temperature-dependence of immunity development (1)

Study protocol:

- A. salmon held at 2, 4 or 8°C were immunised with a multivalent adjuvanted bacterin
- After 6 weeks, fish from each immunisation group was adjoined for challenge (3 parallel tanks)
- Exposure by waterborne infection
- Same procedure was repeated 12 weeks after immunisation

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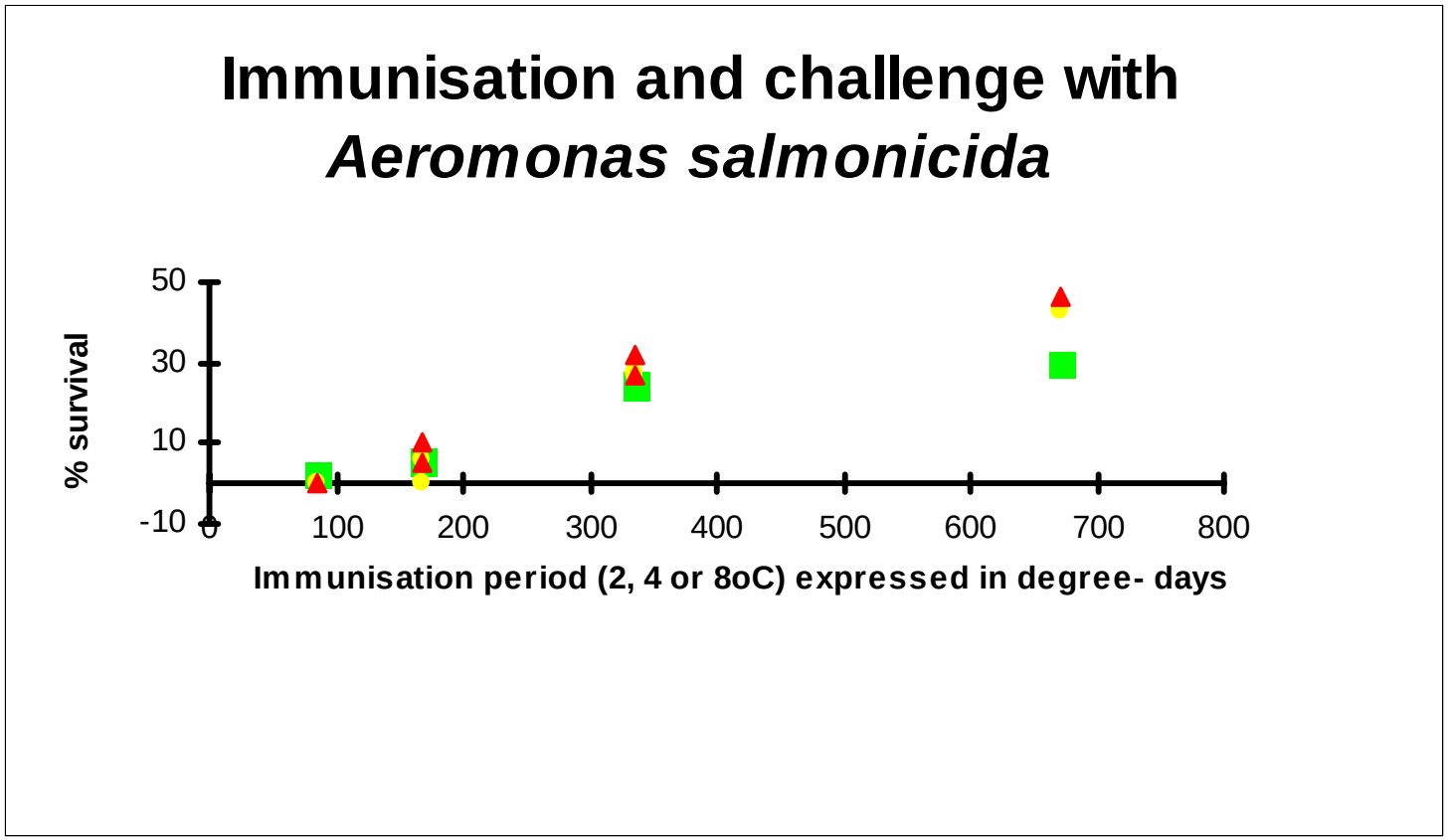
Temperature-dependence …(2), the concept of ”degree-days”



Slow onset of immunity to furunculosis

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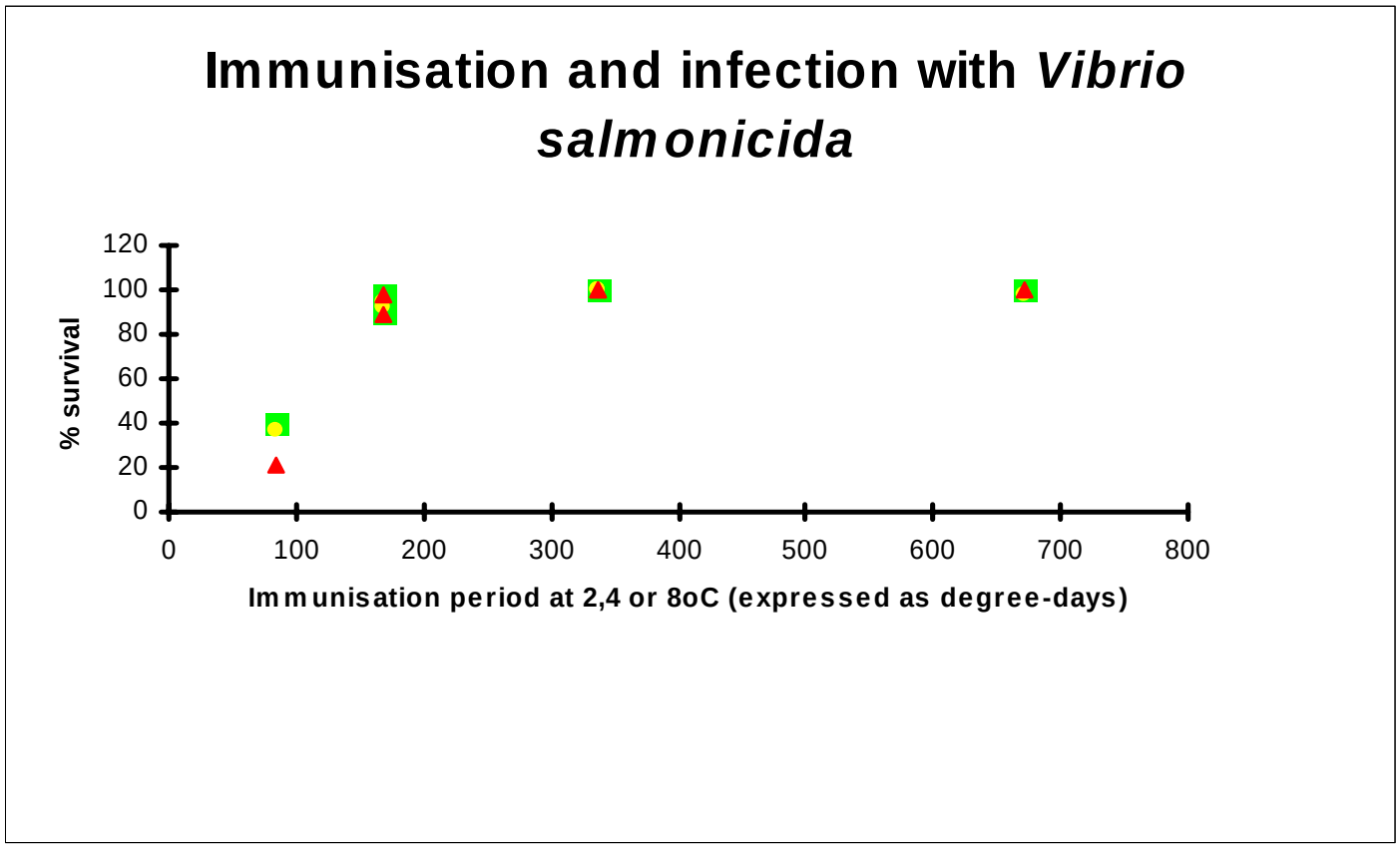
Temperature-dependence of immunity development (3)



Rapid onset of immunity to coldwater vibriosis

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Temperature-dependence of inflammatory reactions

- Local reactions to vaccine emulsions was clearly higher in fish immunised august-september (at water temperatures >12°C) than in fish immunised during winter (at water temperatures <6°C later (Berg et al, Dev. Biol. 121, p. 294, 2005)

Local side-effect studies conducted under too low water temperatures may not reveal the full harmful potential of the formulation

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Limited repertoire of assay reagents for fish immunology

- Anti-fish-immunoglobulin MAbs are poorly characterised
- Few immune cell markers available
- Only few signalling molecule genes described

Due to the limited size of the ”fish biomedical research market”, this situation will likely prevail , or improve very slowly

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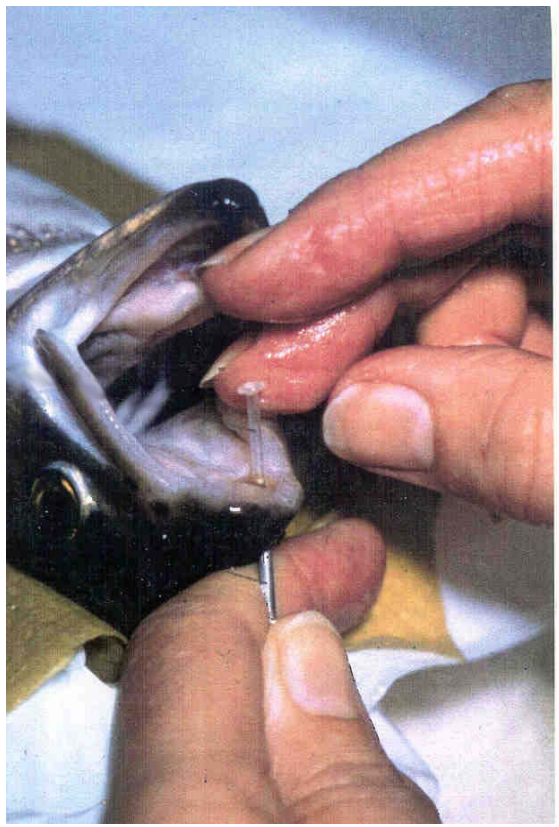
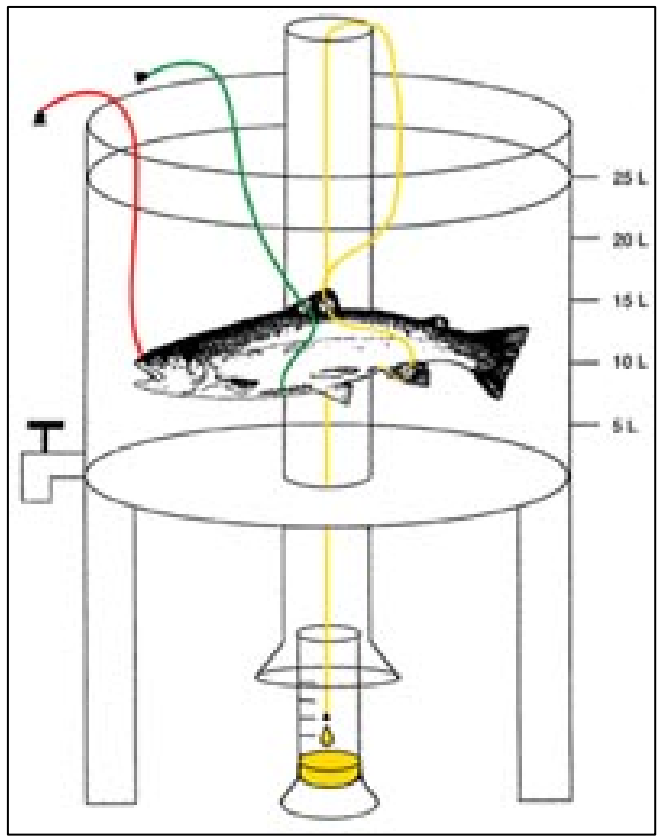
It is difficult for the scientist to work under water





Innovative aorta, bile duct and ureter cannulation by Sohlberg, (Ph.D Thesis NSVS 1998). Faeces collection still a problem

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Fish are ”unstandardised” experimental animals

- Poor access to documented SPF stocks
- Serology of doubtful value most parts of the year
- Size differences develop rapidly under normal rearing conditions
- (Mostly) no external sex determination possible

High response variability combined with small group sizes – an impossible combination

Long-term site-/population surveillance for pathogens, combined with serosurveillance and strict zoo-sanitary management required for proof of SPF status

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Genetically determined resistance to disease – an significant factor

- Innate resistance to several bacterial, viral and even parasitic infections of fish has shown a moderate to high heritable genetic component
- Likely associated with lack of domestication and short history of systematic breeding

Unknown genetic factors may interfere strongly with infection trials in fish

Mortality of Atlantic salmon families after IPN virus challenge

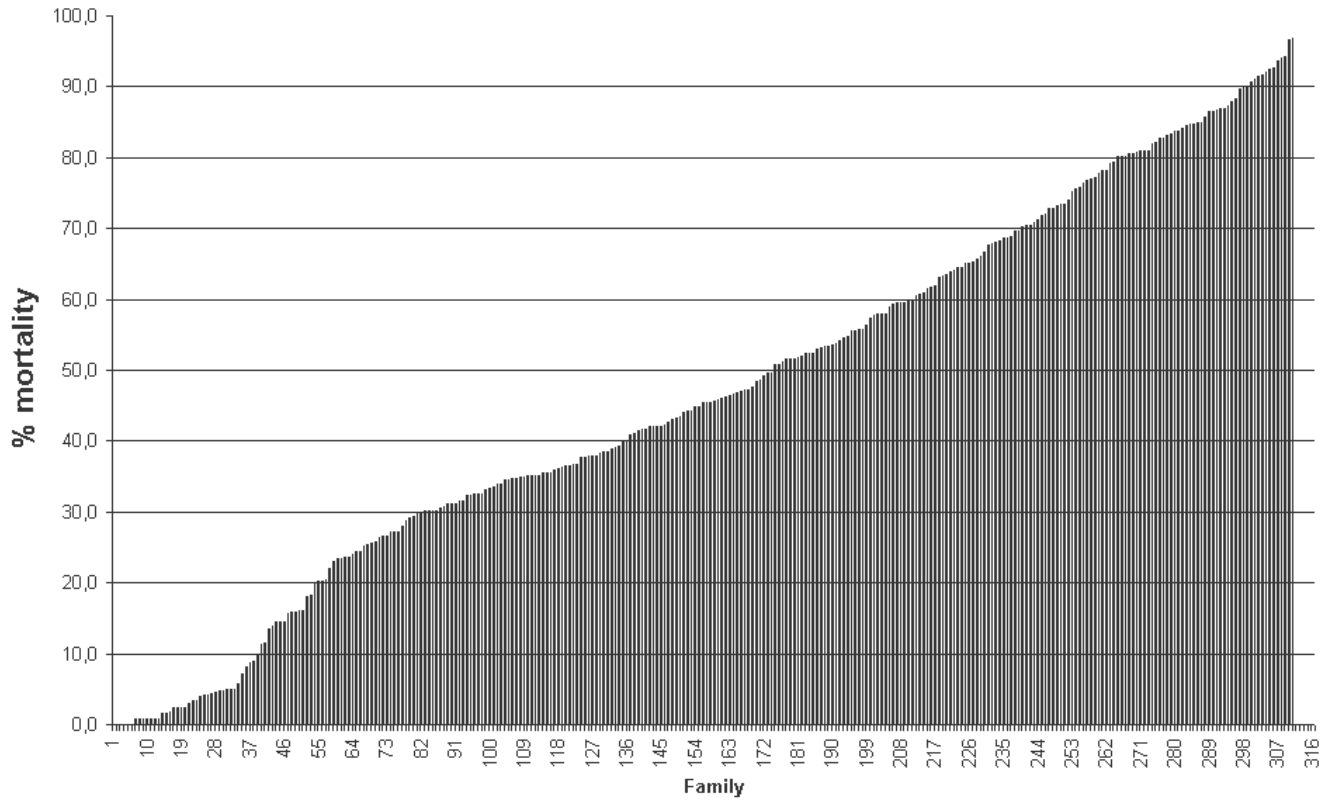

Figure courtesy of AquaGen AS

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Interference of fish yearclass with the outcome of challenge trials

- Identically designed IPN virus challenge trials were conducted to assess seasonal influence and to validate the use of ”out-of-season” smolts
- Fish was immunised and smoltification was induced by light manipulation
- Bath challenge with virus culture supernatant immediately upon sea transfer

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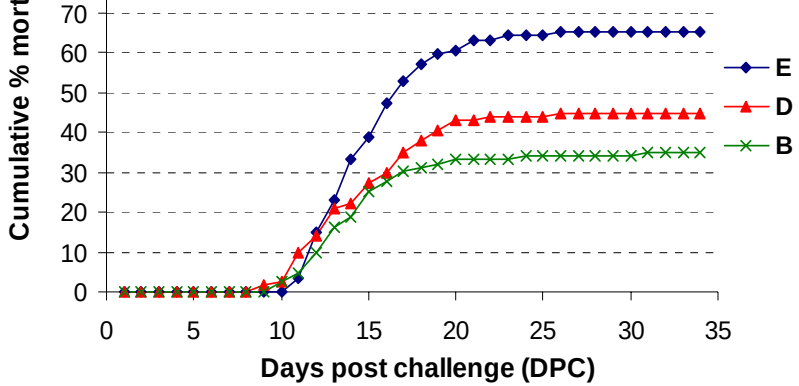
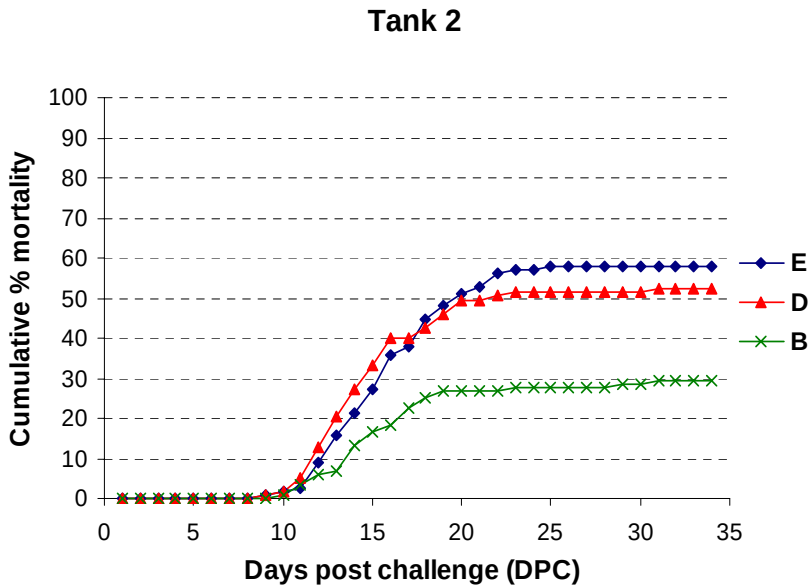
Results 1. challenge (April)





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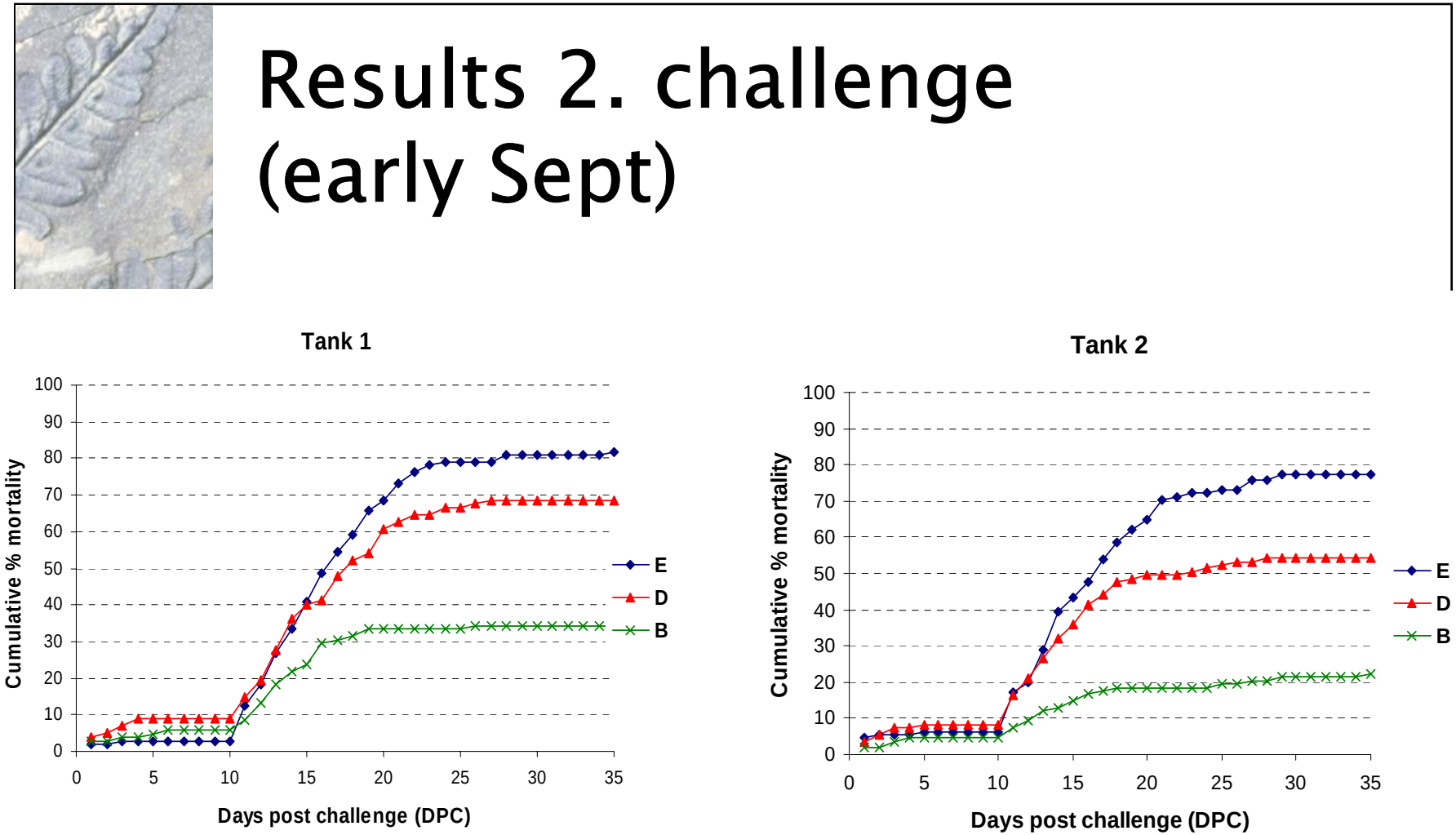
Results 2. challenge (early Sept)



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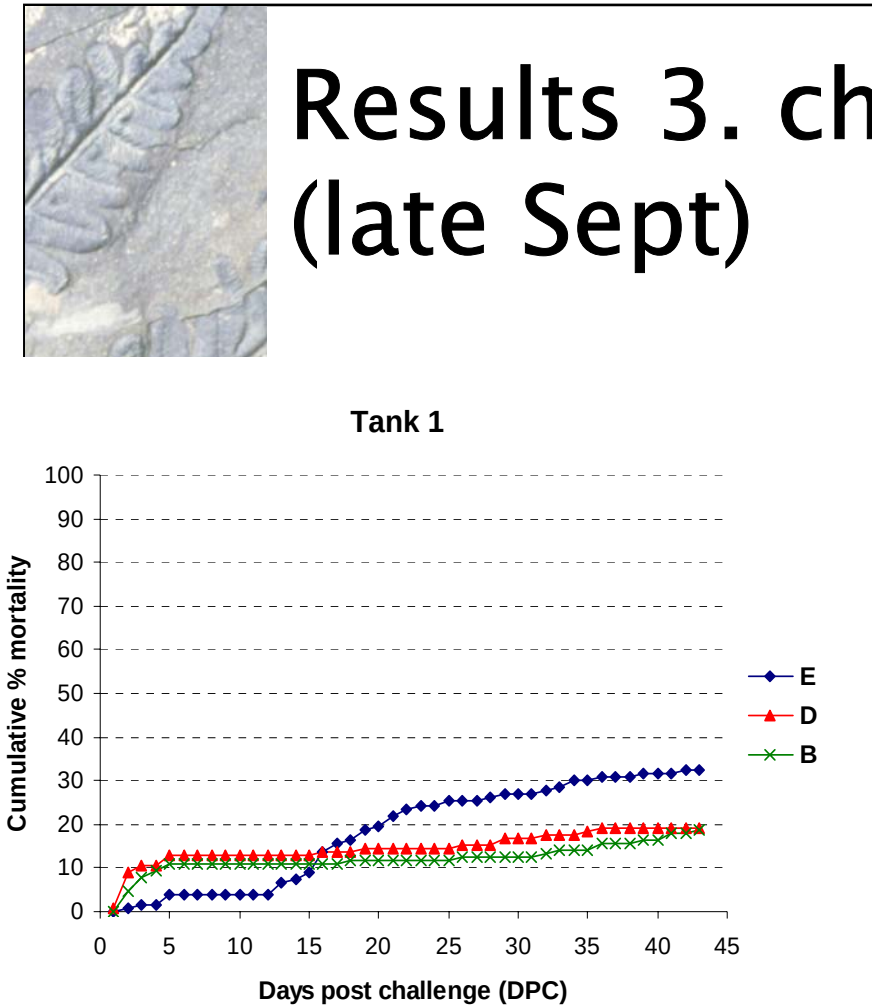
Results 3. challenge (late Sept)



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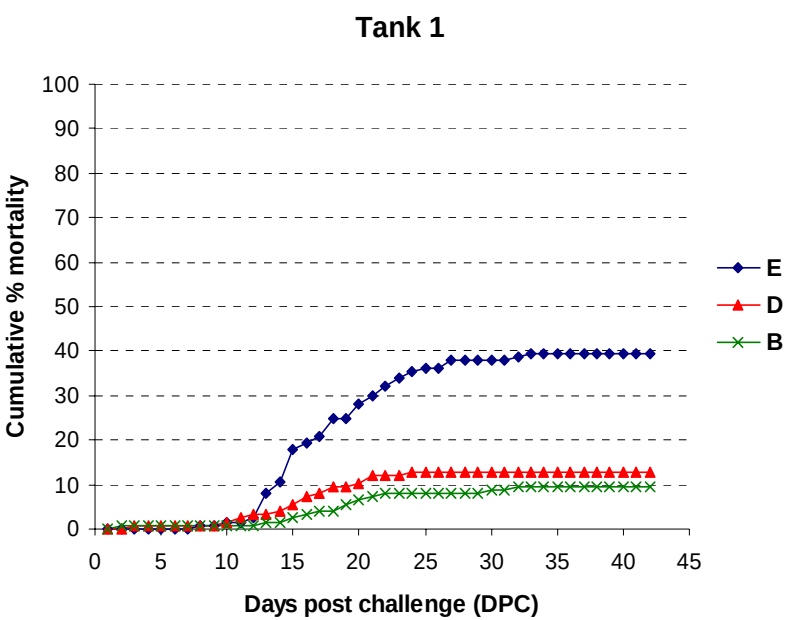
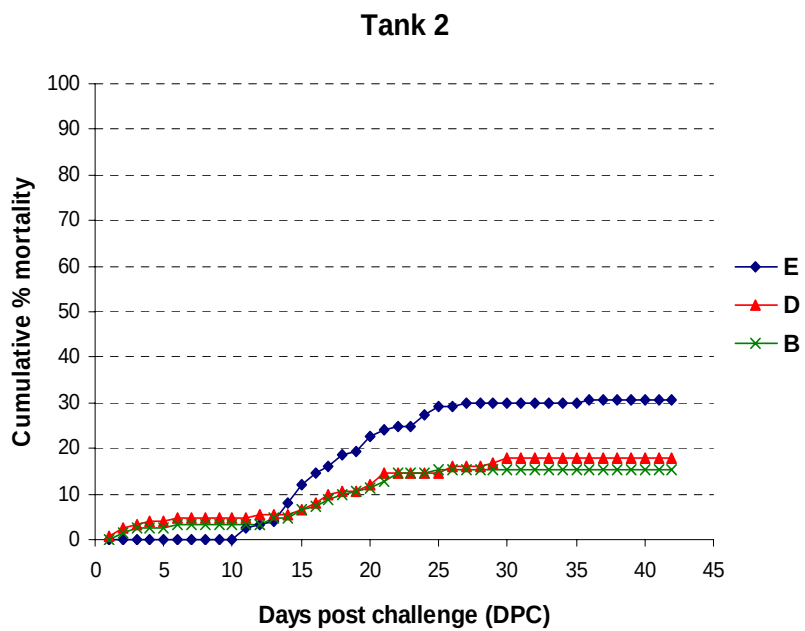
Results 4. challenge (Dec)





Dominant factor for challenge success: smolt yearclass

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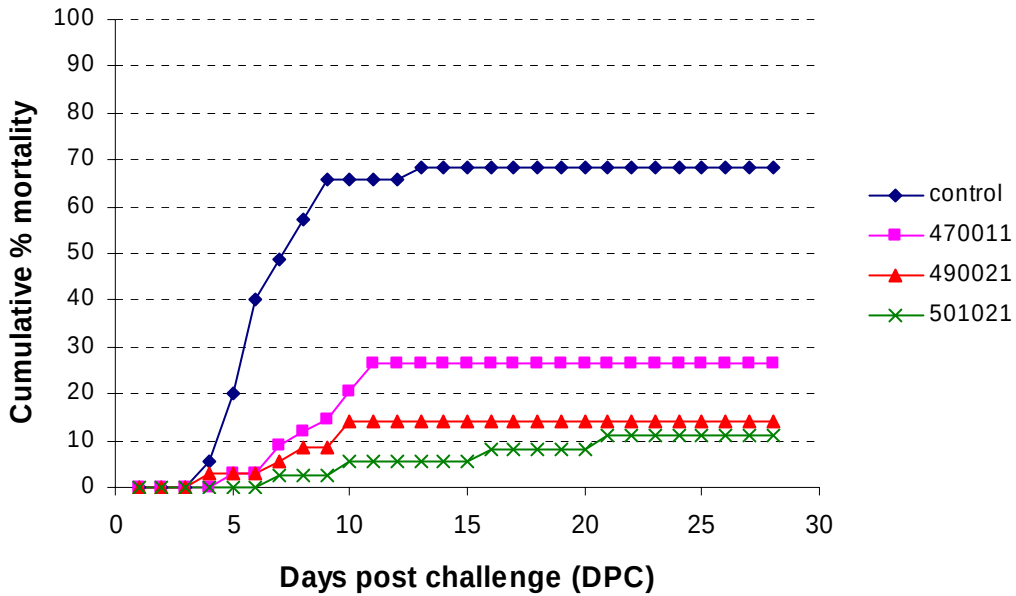
Route of challenge exposure is essential in vaccine efficacy studies (1)



3 winter ulcer vaccine formulations for A. salmon were apparently protective when tested in an i.p. challenge model

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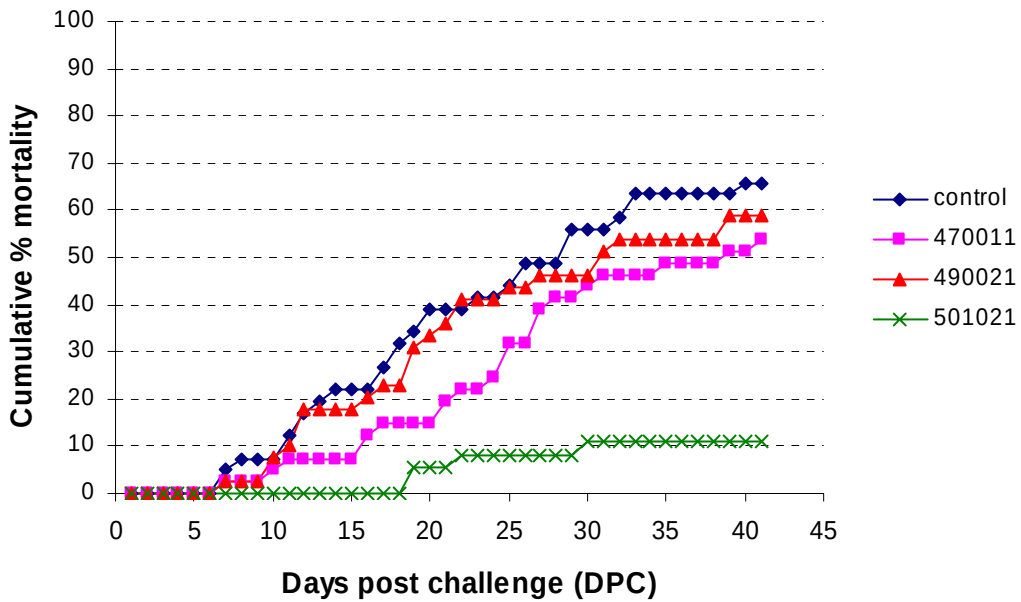
Route of challenge exposure is essential in efficacy studies (2)



Only one of the experimental formulations proved protective when tested in a bath challenge model

Figure courtesy by Pharmaq AS

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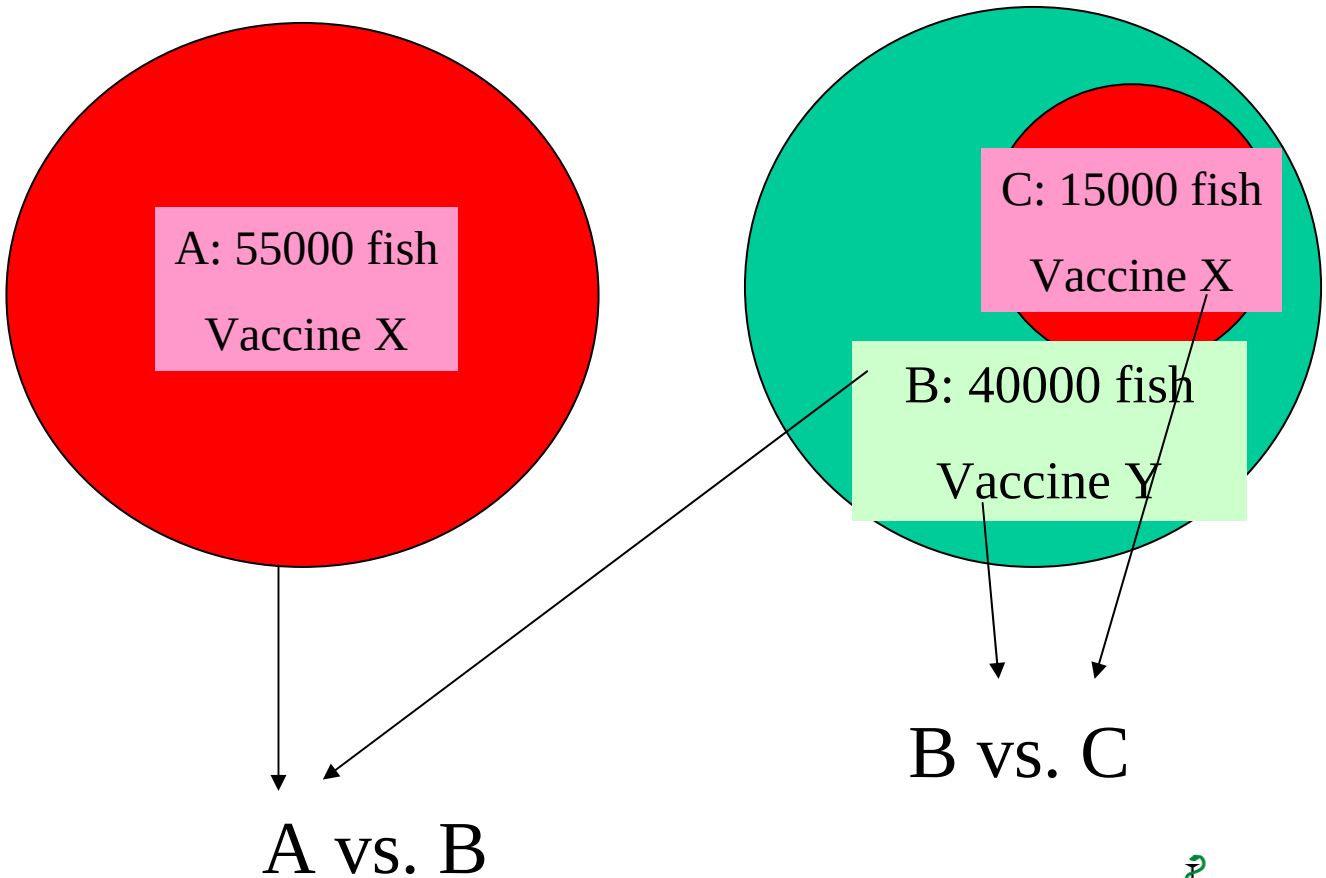
The ”herd effect” in fish trials (1)



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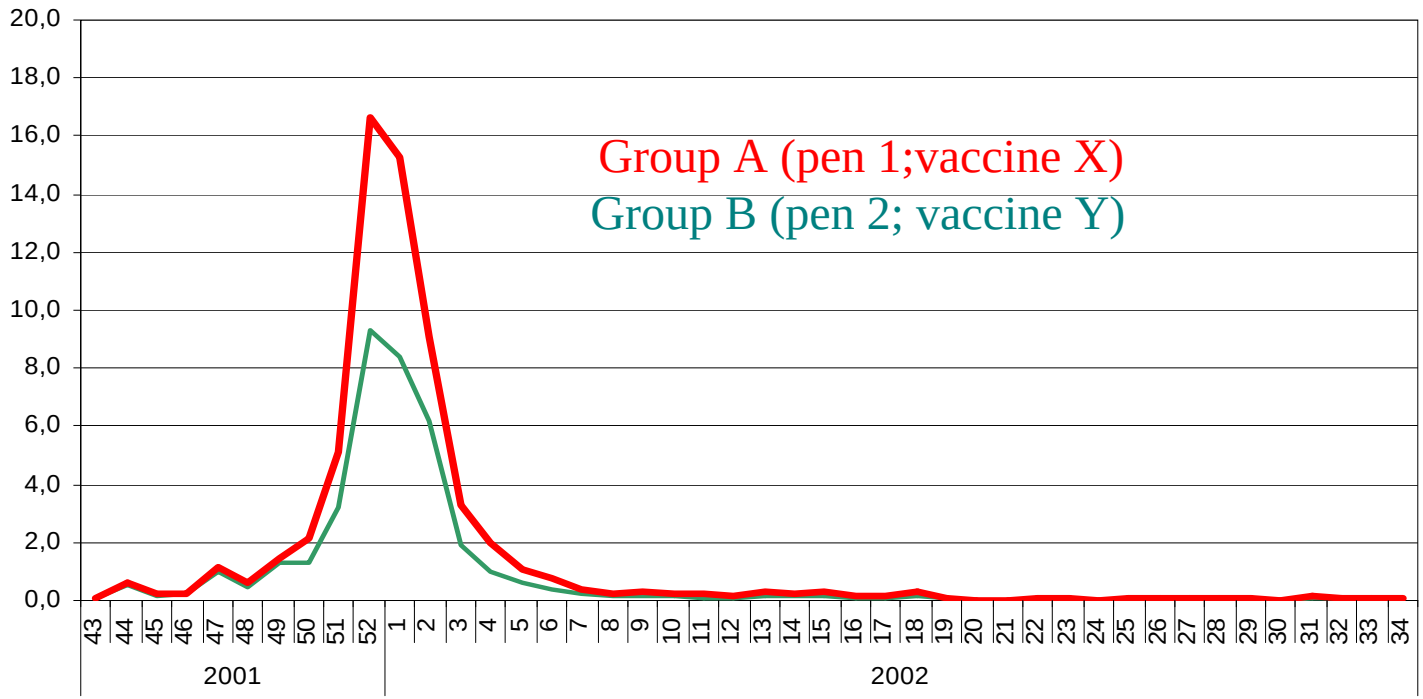
Weekly mortality



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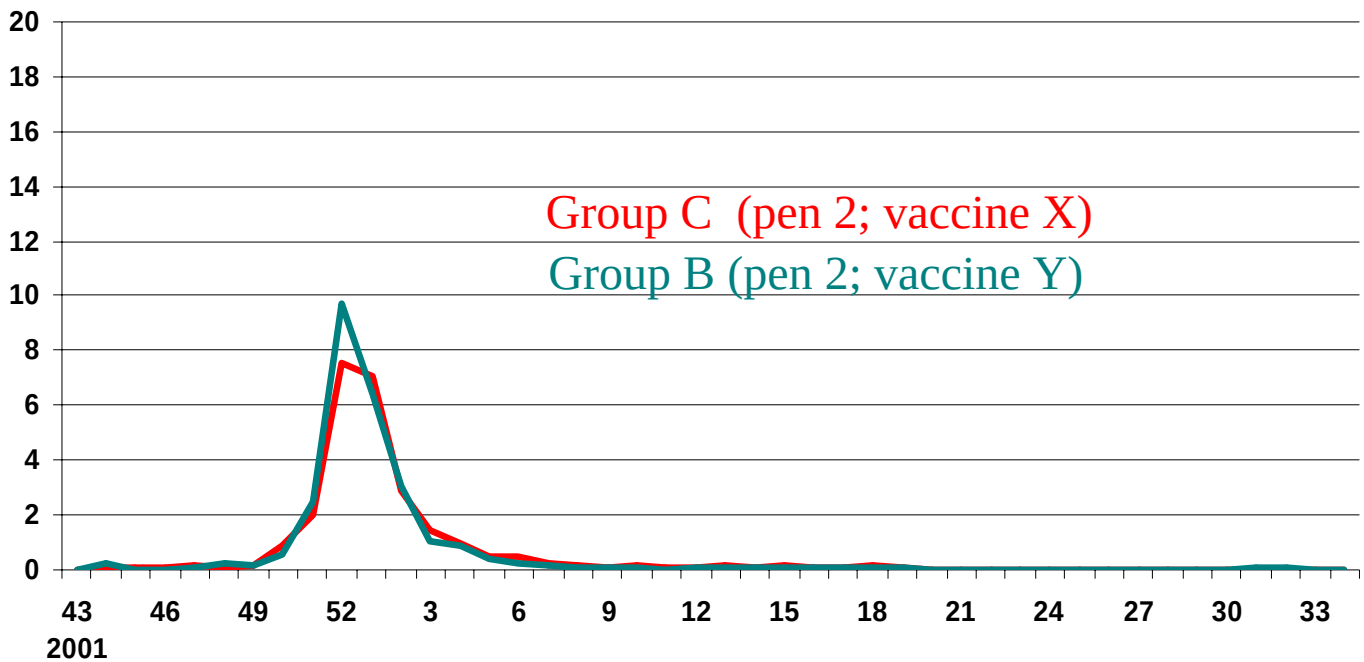
Weekly mortality



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The “pen effect” – results analysis

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
A	44	0	0,120	0,0600	0,505
B	44	0	0,0600	0,0150	0,170

T = 2217,500 n(small)= 44 n(big)= 44 (P = 0,031)

The difference in the median values between the two groups is greater than would be expected by chance; **there is a statistically significant difference (P = 0,031)**

Mann-Whitney Rank Sum Test

Group	N	Missing	Median	25%	75%
B	44	0	0,0600	0,0150	0,170
C	44	0	0,0650	0,0300	0,180

T = 1909,500 n(small)= 44 n(big)= 44 (P = 0,689)

The difference in the median values between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability**; there is not a statistically significant difference (P = 0,689)**

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Further study

VESO has hosted and organised two major scientific conferences on fish immunology and vaccination:

- 2nd IABs Symposium on Fish Vaccinology, Oslo June 1-4, 1996
  - "Fish Vaccinology": Dev. Biol. Stand. 90; 464 pp. (1997).
- 3rd IABs Symposium on Fish Vaccinology, Bergen, April 9-11, 2003
  - "Progress in Fish Vaccinology: Dev. Biol. 121; 340 pp. (2005).
- A few copies of these books still available at VESO, alternatively to be purchased from the IABS (www.iabs.org).

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Thank you for your attention!

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