Norwegian School of Veterinary Science

SALMAR

Vaccination and spinal deformities
- associations and causal mechanism

Arnfinn Aunsmo

BVM&S (1994)

PhD student 2005-2008

Norwegian School of Veterinary Science

Centre for Epidemiology and Biostatistics

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Manager Biology and Nutrition

Contents

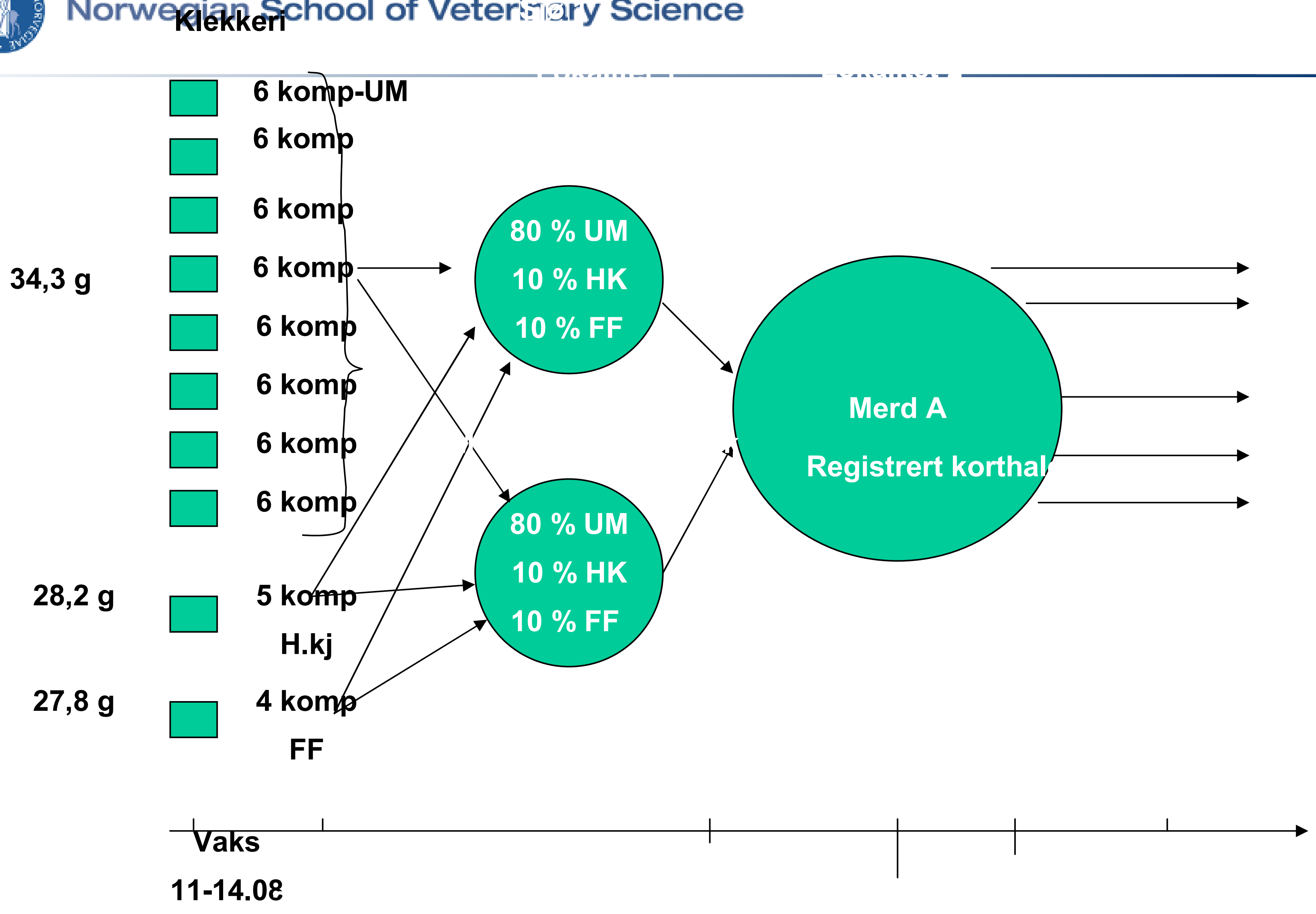
- Literature
- Studies
  - A. Aunsmo, A. Guttvik, P.J. Midtlyng, R.B. Larssen, Ø. Evensen, E. Skjerve, 2008. **Association of spinal deformity and vaccine induced abdominal lesions in harvest sized Atlantic salmon, *Salmo salar.*** Journal of Fish Diseases 31: 515-524
  - A. Aunsmo, S. Øvretveit, O. Breck, P.S. Valle, R.B. Larssen, M. Sandberg. **Modelling sources of variation and risk factors for spinal deformity in farmed Atlantic salmon using hierarchical and cross-classified multilevel models.** Preventive Veterinary Medicine (submitted 2008).
  - A. Aunsmo, R.B. Larssen, P.S. Valle, M. Sandberg, Ø. Evensen, P.J. Midtlyng, A. Østvik, E. Skjerve, 2008. **Improved field trial methodology for quantifying vaccination side-effects in farmed Atlantic salmon (*Salmo salar* L.)**. Aquaculture 284: 19-24.
- Causal mechanism

Literature

- Vaccination and side-effects, oil adjuvanted vaccines
  - Paul Midtlyng, several papers (1996-1998)
  - ”Speilberg score” for vaccine induced intra abodominal lesions
  - Growth retardation associated with high lesion scores
- Vaccination and spinal deformity
  - Case reports (Waagbø et el 2005)
  - Epidemiological study (Djupvik and Larssen 2005)
- Association between no of deformed vertebrae and high lesion scores (Berg et al. 2006)

Association of spinal deformity and vaccine induced abdominal lesions in harvest sized Atlantic salmon

- Vaccine trial VESO Vikan (efficacy trial)
- 3 vaccine groups in cohabitant fish
  - 4,5 and 6 components
- Spinal deformity observed in one of the vaccine groups
- Preharvest samples
- Samples at harvest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Harvest results

- Harvest weight 4,5 kg
- Superior quality 87%
- Production quality 12%
  - Spinal deformity
- Weight deformed fish 2,8 kg

Study results

	4 comp	5 comp	6 comp
SGR after vacc*	1,90	1,60	1,20
Preharvest sample** no. normal (deformed)	89 (0)	42 (0)	455 (75)
Harvest sample no. Normal (deformed)	83 (2)	69 (7)	148 (200)
Adhesion score Normal (deformed)	1,7 (2,5)	2,1 (2,6)	2,2 (2,9)
Round weight kg Normal fish	5,5	4,8	4,7

* 20.08-27.09

** Random sample

”Short tails”

Lesions more cranially

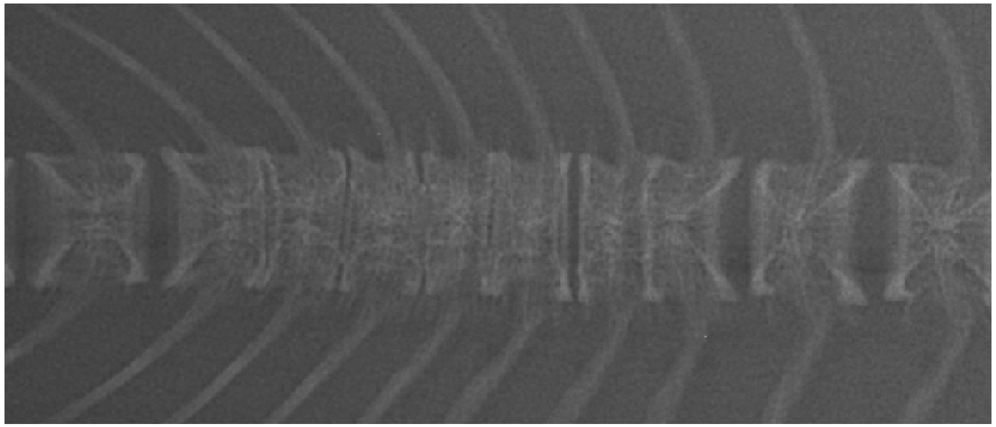
Radiography

24 visually deformed fish

- Extensive vertebral body compression
  - On average 26 per fish
  - Tail region
    - 16 fish
  - Both tail ang thoracic region
    - 4 fish
  - Whole vertebral column
    - 4 fish
- Vertebral fusion (13 fish)

25 visually normal fish

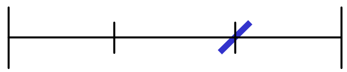
- 2 fish with lesions
- 5 and 16 compressed vertebrae
- 3 and 11 fused vertebrae



Outcome measurements and recordings

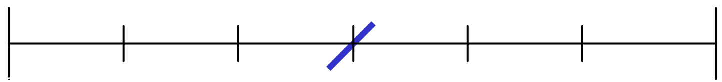
- **Weight in 5 gram (ungutted)**
  - **Total length in 0,5 cm**
  - **Mark (UM, AF or RM)**

- **Deformity score (VAS)**

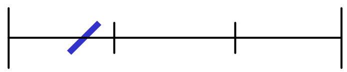


- **Opening abdominal cavity**

- **Adhesion score / ”Speilberg score” on a VAS (modified after Midtlyng et al. 1996)**



- **Melanin score (VAS)**



Statistical analysis

Multivariable logistic regression model associating markers of vaccine side effects to spinal deformity in the case-control sample. Odds ratios are shown for one unit increase in the continuous variables adhesion score (0-6) and melanin on abdominal organs (0-3) and for presence of lesions in caudal dorsal abdomen

Variable	coefficient	S.E.	OR	95% CI (OR)	P
Intercept	-7.32	0.64	-	-	-
Adhesion score (0-6)	1.73	0.26	5.65	3.39, 9.41	<0.001
Melanin on abdominal organs (0-3)	1.60	0.38	4.93	2.33, 10.43	<0.001
Lesions in caudal dorsal abdomen	0.81	0.27	2.24	1.31, 3.82	0.003

Summary

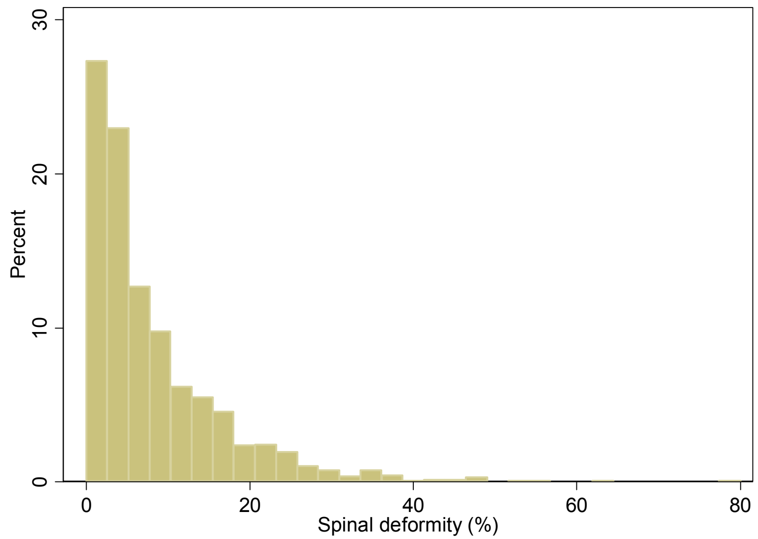
- 14% spinal deformity in one vaccine group
  - Vaccine batch with ”high side-effect scores”
  - Very few in 4 and 5 component groups
- Lesions both in tail region and more cranially
  - Compressed and fused vertebrae
- Increased odds for spinal deformity with increased lesion scores
- Effect on harvest weight

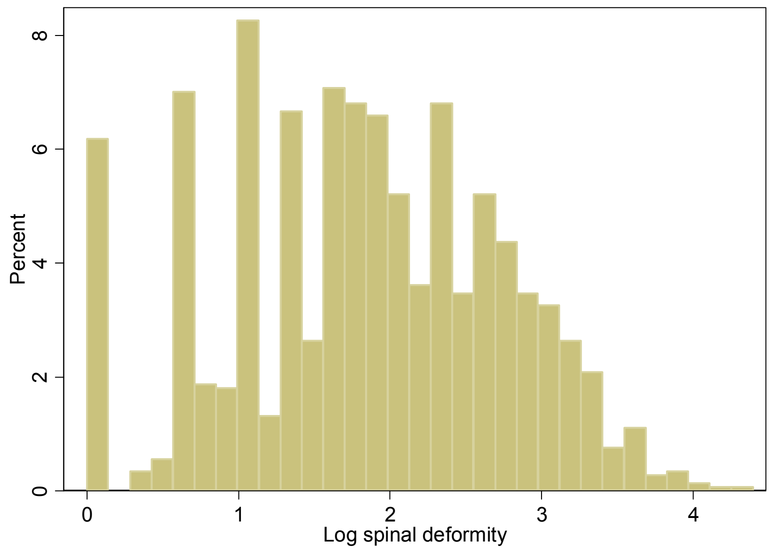
Variance components and risk factor for spinal deformity – multilevel modelling

- Dataset Marine Harvest Norway (MHN)
- Generation 2002-2004
- 2 regions, 5 counties
- Close to 30 mill fish harvested

Spinal deformity – outcome variable

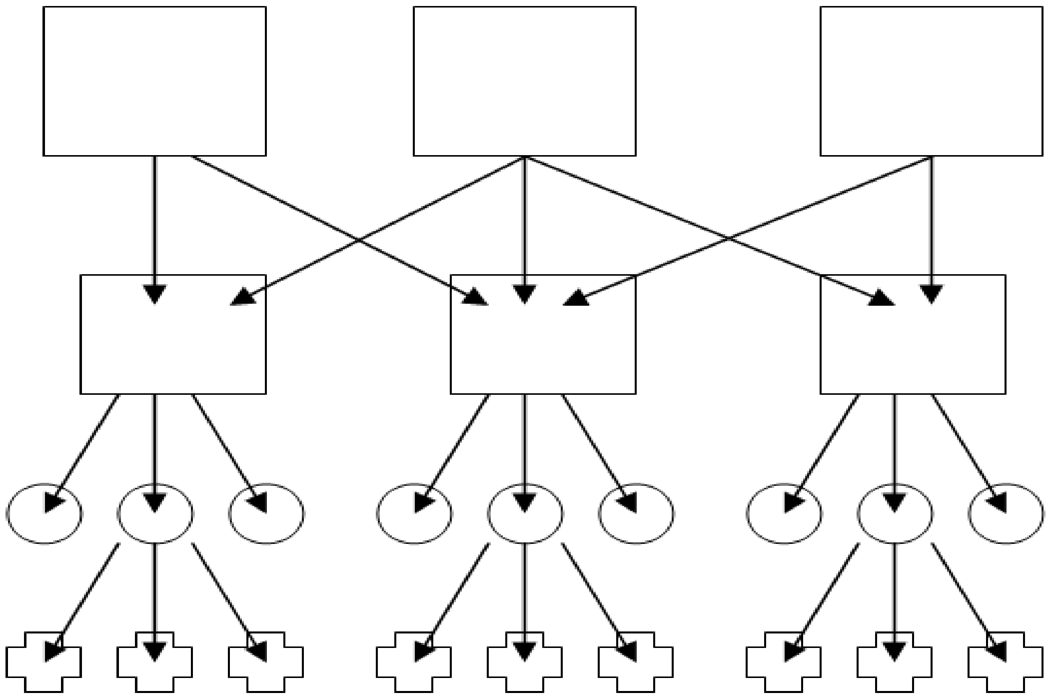
- Daily subsamples at the harvest line
  - Average 118 fish/ harvest day, 170 000 fish total
- Spinal deformity yes/no
- Aggregated as prevalence spinal deformity
  - Average 8% (0-80%)
- Logtransformed outcome variabel (normal distribution)





Data structure (MHN)

a combined hierarchical (nested) and cross-classified datastructure

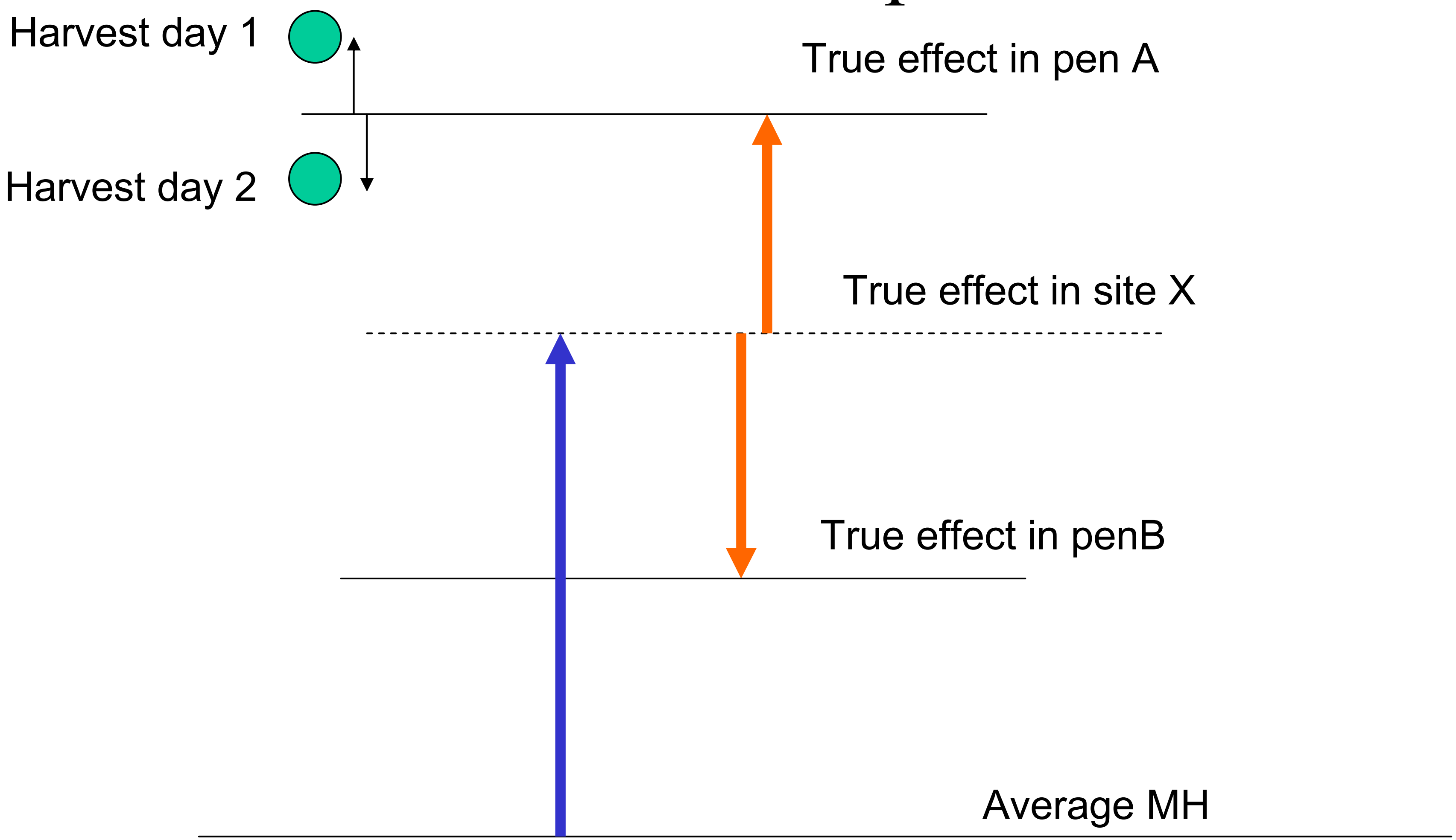


- Fresh water plants (n=21)
- Sea sites (n=39)
- Pens (n=544)
- Harvest days (n=1441)

Variance components

- Where do variation arise?
  - Total variance decomposed to each level in the production hierarchy
  - Identify levels where intervention is most likely to be productive
- Variation in an empty model
  - No fixed effects (explanatory variables)
- Variation in a ”mixed effect model”
  - Including fixed effects

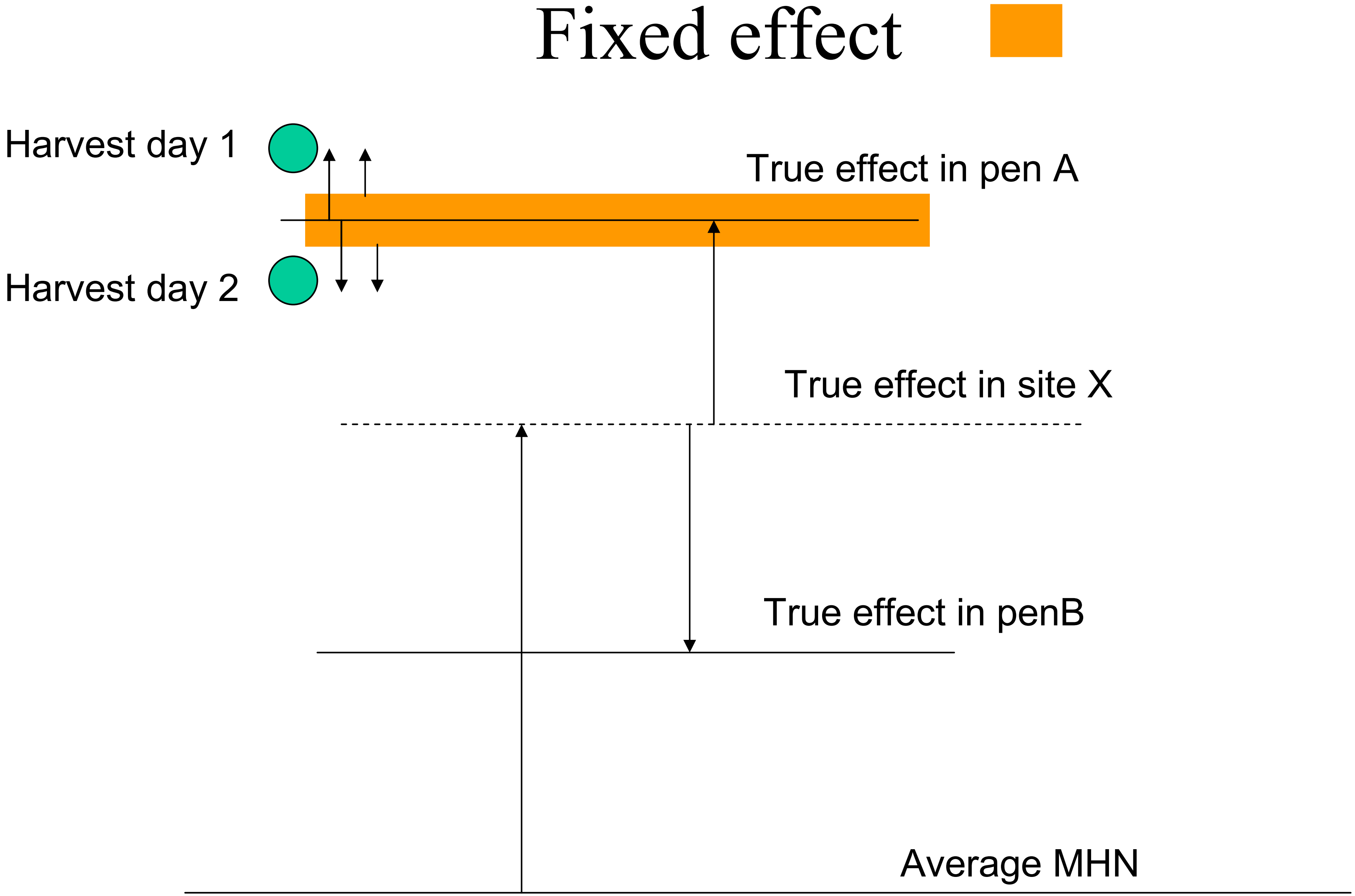
Random effects
-variance components



Fixed effects (risk factors)

- Factors explaining the variation

Fixed effect



Statistical analysis

- Challanging! – limitations in software
  - Especially cross-classified models
- Clustering effect
  - Fish with pens are more like than between pens
  - Not indedendent data!
- MLwiN (Multilevel windows)
  - Developed for multilevel analyses
- Markov chain Monte Carlo estimation (MCMC)
  - Cross-classified models
  - Model comparison
  - DIC (Bayesian Deviance Information Criterion)
- Restricted iterative generalized least square (RIGLS)
  - For hierarchical (nested) multilevel models
  - Model comparison -2loglikelihood,

Results

- Variance components
- Risk factors

Variance components

Level	Empty model (% av total variance)	Mixed modell (% av total variance)	Variance component reduction (%)
Fresh water plant	0,12 (14%)	0,10 (15%)	17 %
Sea water site	0,34 (38%)	0,18 (25%)	48%
Pen	0,14 (16%)	0,13 (19%)	7%
Harvest day	0,28 (32%)	0,28 (41 %)	0
Total variance	0,88 (100 %)	0,69 (100 %)	22%

Risk factors

Uni-variabel screening (=t-test)

Variable	mean /cat	Min	Max	P-value
Breed	4			<0,001
Breed2	2			<0,001
Smolttype	3			<0,001
Vaccine	10			<0,001
Vacc.prod	3			0,03
Region	2			0,9
County	5			<0,001
Year	3			<0,001
Min weight vacc	52,2 g	29	170	<0,001
Dead 1st month	1,8%	0,05	30,2	<0,001
Temp vacc	10,6 gr.C	2	19,5	<0,001
Weight sea trans	93,6 gr	47,8	320	<0,001

Risk factors for spinal deformity ”mixed effect model”

Fixed effects	β	S.E.	95% C.I.
Intercept	1.006	0.059	0.89, 1.12
S0.5*	0.762	0.122	0.52, 1.00
S1.5*	0.343	0.166	0.02, 0.67
6-comp vacc**	0.239	0.117	0.01, 0.47

***vs. S1**

****vs. 4 and 5 comp vaccine**

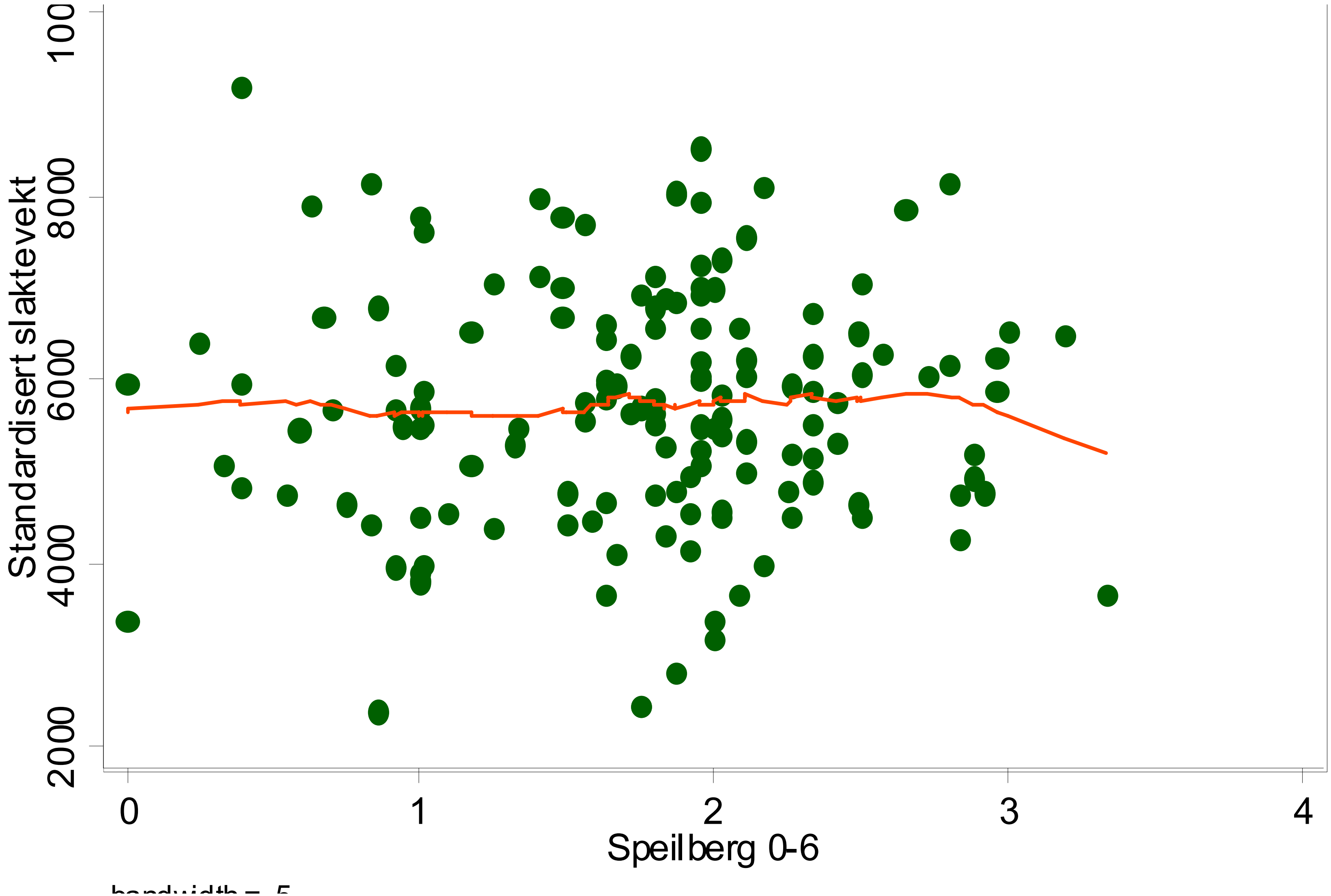
To summerize

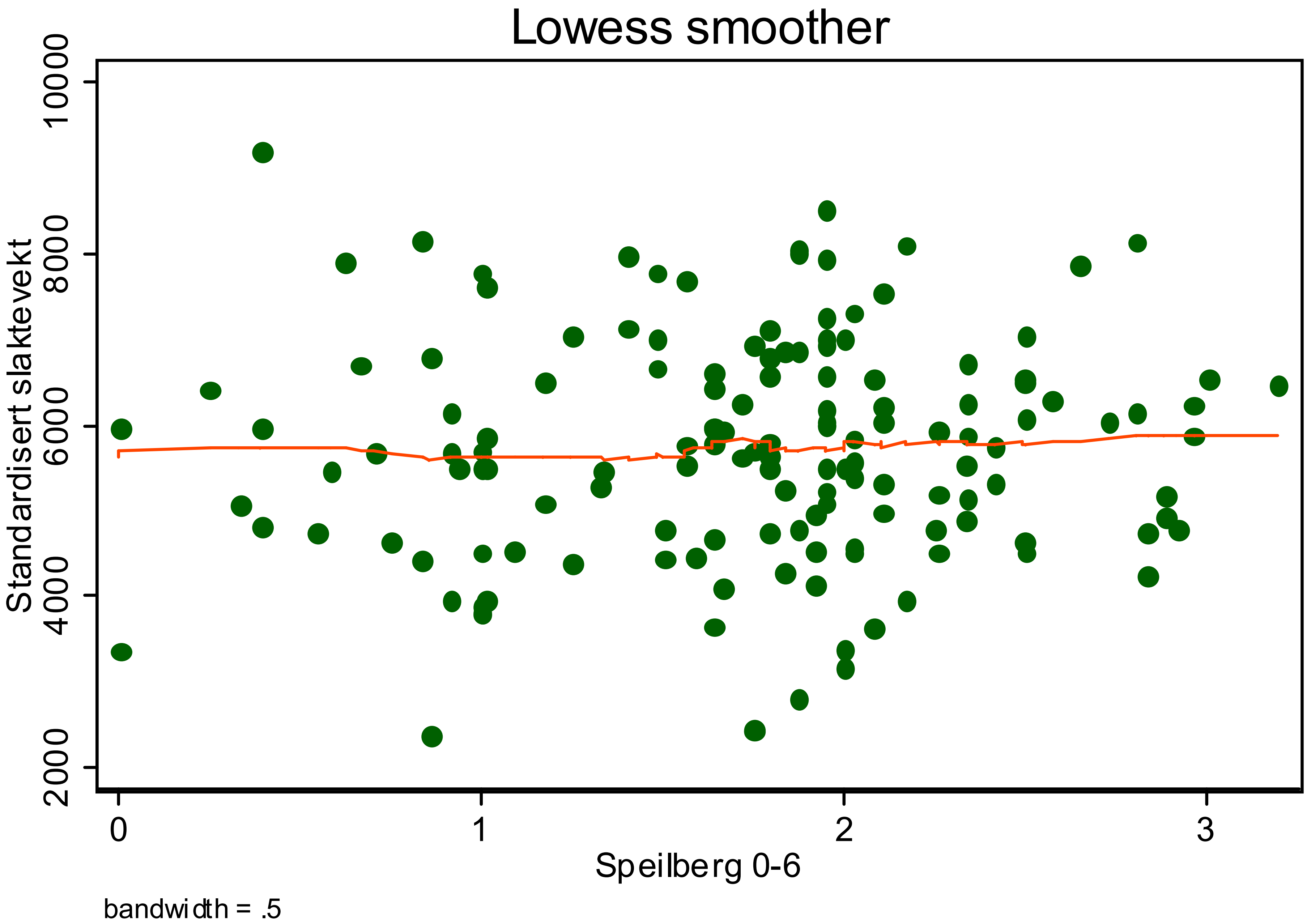
- Automn smoltification is a risk factor for spinal deformity
  - Not only effect of age, also 1,5yr old smolt
- Vaccination is a risk factor
  - Not only specific vaccine batches, general problem
  - Also 5 component vaccines accociated with spinal deformity
  - We do not know total vaccine effect
- Limited variation between fresh water plants

Improved field trial methodology for quantifying vaccination side-effects in farmed Atlantic salmon.

- Individually PIT tagged fish followed from vaccination till harvest, both vaccinated and unvaccinated
- Vaccinated fish 0,5 kg smaller than unvaccinated fish
- No effect of lesion score (Speilberg) on harvest weight.
- Normal fish, very few fish with spinal defomity
  - 1,2 kg reduced harvest weight on 11 deformed fish

Lesion score vs. harvest weight




Lowess smoother
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8000
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1
2
3
Speilberg 0-6
bandwidth = .5

Causal mechanism

- Causal factor
  - ”Any factor that produces a change in the severity or the frequency of the outcome” Dohoo et al. 2003

> Multivariable causation!

- Limited number of mechanisms for spinal deformity
  - Incubation temperature
  - Nutrition
  - Vaccination?

Immunoligical mechanism?

- No association between antibody level and degree of lesion score (AB Romstad pers. com.)
  - Lesion score (Speilberg) not associated with immune response
  - Local reaction
- No association between harvest weight and lesion score
  - Growth in normal fish independent on lesion score
  - (very high lesion scores shown to be associated with weight reduction)
- Same lesion level, different prevalence of spinal deformity
  - More to vaccine associated spinal deformity than lesion score

Nutritional mechanism

- Oil based vaccines cause appetite depression (Sørum and Damsgård 2004)
- Growth of length is enhanced at smoltification (Young et al 1995)
- Increased longitudinal growth of vertebrae caudal to abdominal cavity during smoltification (Fjelldal et al. 2005)
  - Caudal vertabrae are at increased risk
- Nutrional effects on skeletal development and malfomations
- Salmon are vaccinated during smoltification
  - Especially S0s

Nutrional mech. cont.

- Growth of autumn smolitfied smolt is enhanced
  - Increased temperature
  - Growth stimuli in light period
- Fish are starved at vaccination
  - 9 days!
- Additional appetite depprivation by oil-adjuvanted vaccines
  - Peritonitis
  - Antigen specific effect
  - Moritella viscosis AG are more potent (Mutoloki 2007)

Mechanism cont.

- Threshold for effect on vertebral development?
- ”Collapse” when mineraltization reaches a minimum level.
- Dramatic effect on skeletal development, growth etc.

Conclusion

- ”All” factors affecting feed uptake have the potential of causing skeletal malformations!!
- Vaccination is a very potent regulator of feed uptake
  - Starving
  - Appetite depprivation