

Sea Lice



Resistance Management – an update on R&D

Tor E. Horsberg

Norwegian School of Veterinary Science



Treatments used in Norway

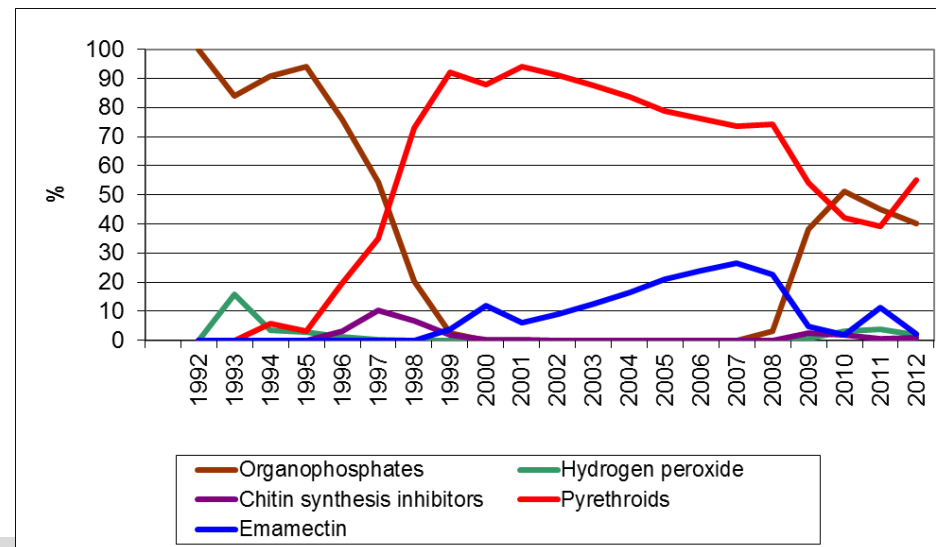


- Consumption data are publically available

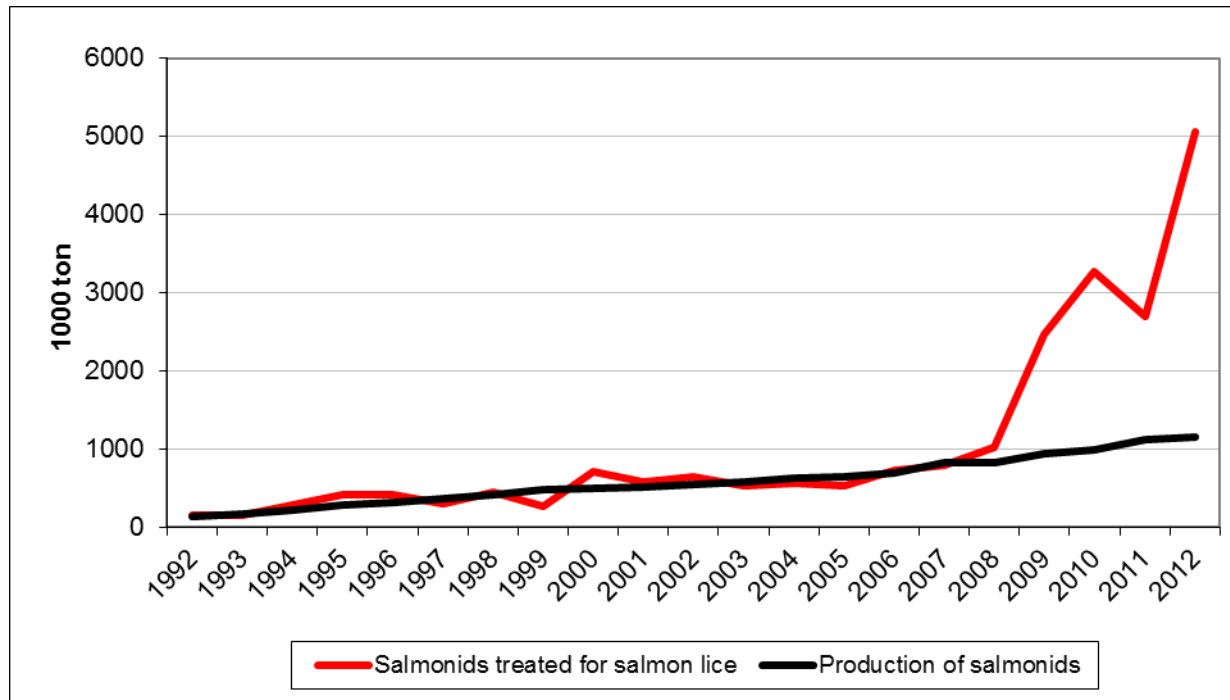
CONSUMPTION (kg)	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
metrifonate	1 946	1 779	1 227	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
dichlorvos	3 115	2 470	1 147	395	161	36	0	0	0	0	0	0	0	0	0	0	0	0	0	0
azamethiphos	0	0	389	738	606	315	182	14	0	0	0	0	0	0	0	0	66	1 884	3 346	2 437
H2O2	0	710 000	290 000	340 000	160 000	20 000	0	0	0	0	0	0	0	0	0	0	0	308 000	3 071 000	3 144 000
diflubenzuron	0	0	0	0	160	361	437	50	12	0	0	0	0	0	0	0	0	1 413	1 839	704
teflubenzuron	0	0	0	0	610	1 510	1 334	231	62	28	0	0	0	0	0	0	0	2 028	1 080	26
pyrethrins	0	0	32	26	9	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0
cypermethrin	0	0	0	0	23	28	3	19	73	69	62	59	55	45	49	30	32	88	107	48
deltamethrin	0	0	0	0	0	0	19	11	23	19	23	16	17	16	23	29	39	62	61	54
emamectin	0	0	0	0	0	0	0	4	30	12	20	23	32	39	60	73	81	41	22	105

Torrissen et al. 2013, J Fish Dis

- Relative use of treatments

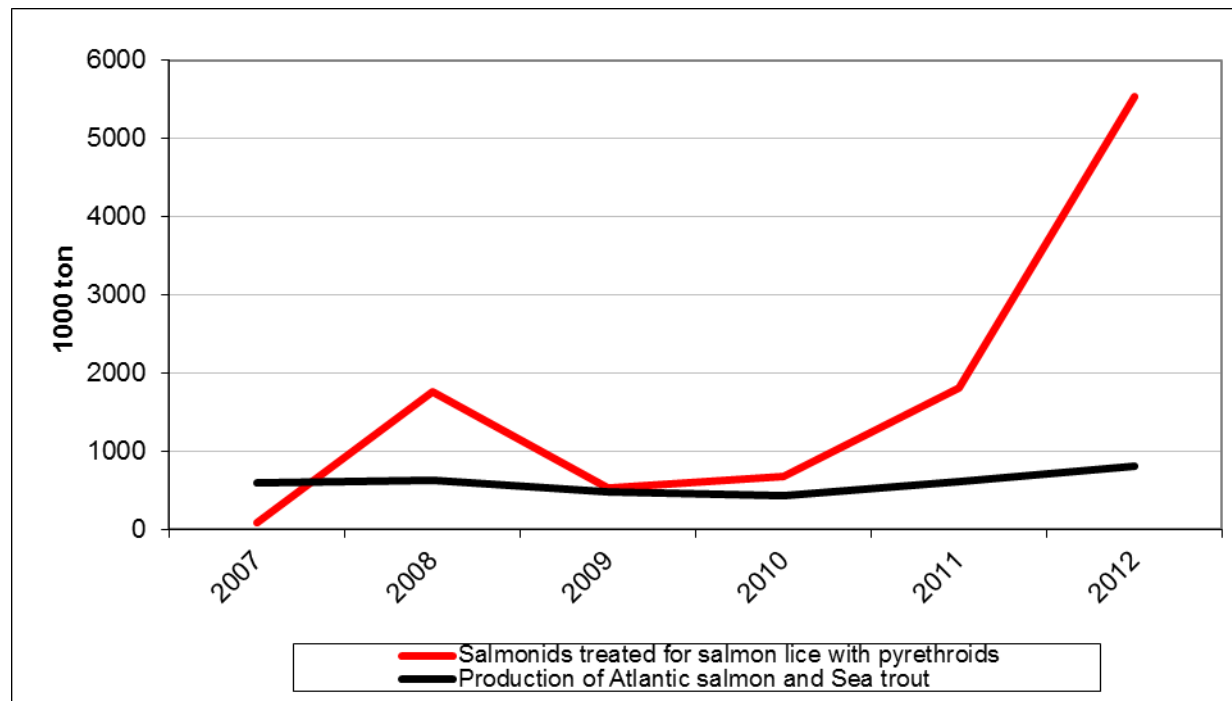


Total use of treatments in Norway



Norwegian Institute of Public Health; Statistics Norway

Total use of pyrethroids in Chile



Sandra Bravo, personal communication

Key points in resistance management

- Surveillance of resistance
 - Diagnostic methods
- Rotation of treatments
 - More than one effective treatment
- Utilization of non-medicinal control options
 - Non-medicinal control options available
- Protection of sensitive parasites!

DIAGNOSTIC METHODS



- Treatment efficacy



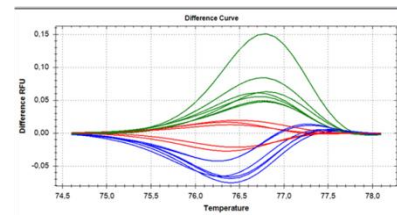
- Full 5-dose bioassay



- Simplified 2-dose bioassay

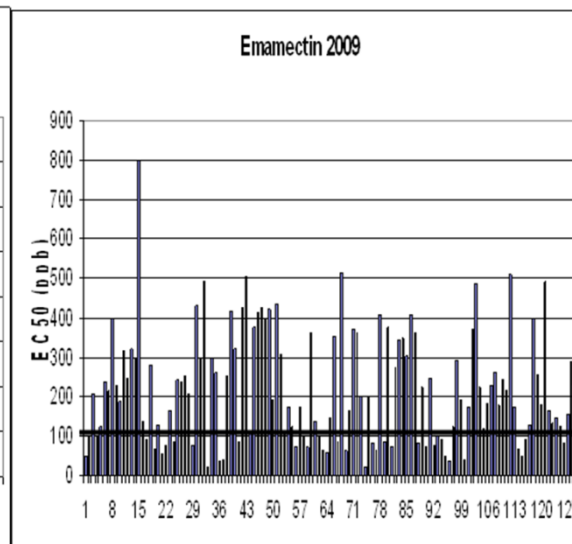
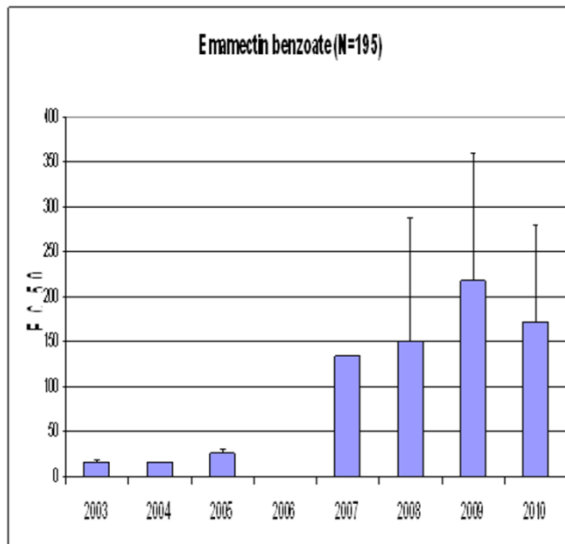
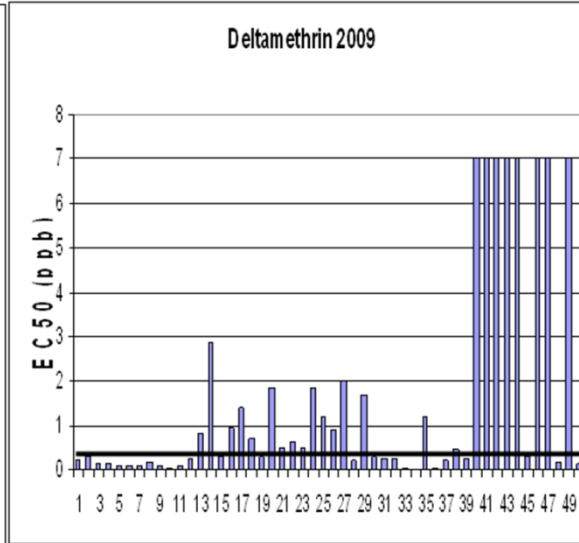
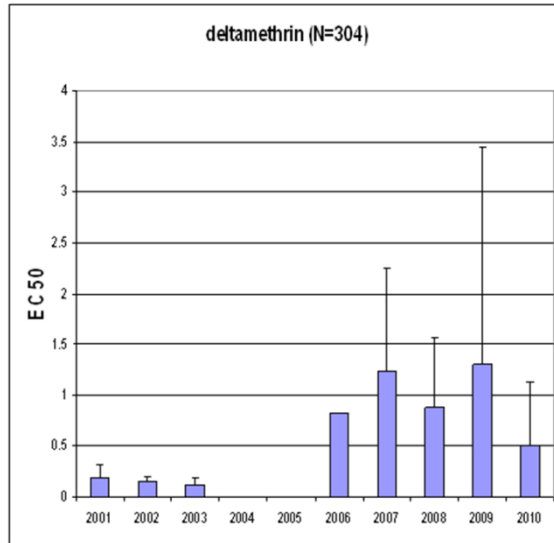


- Biomarker assay



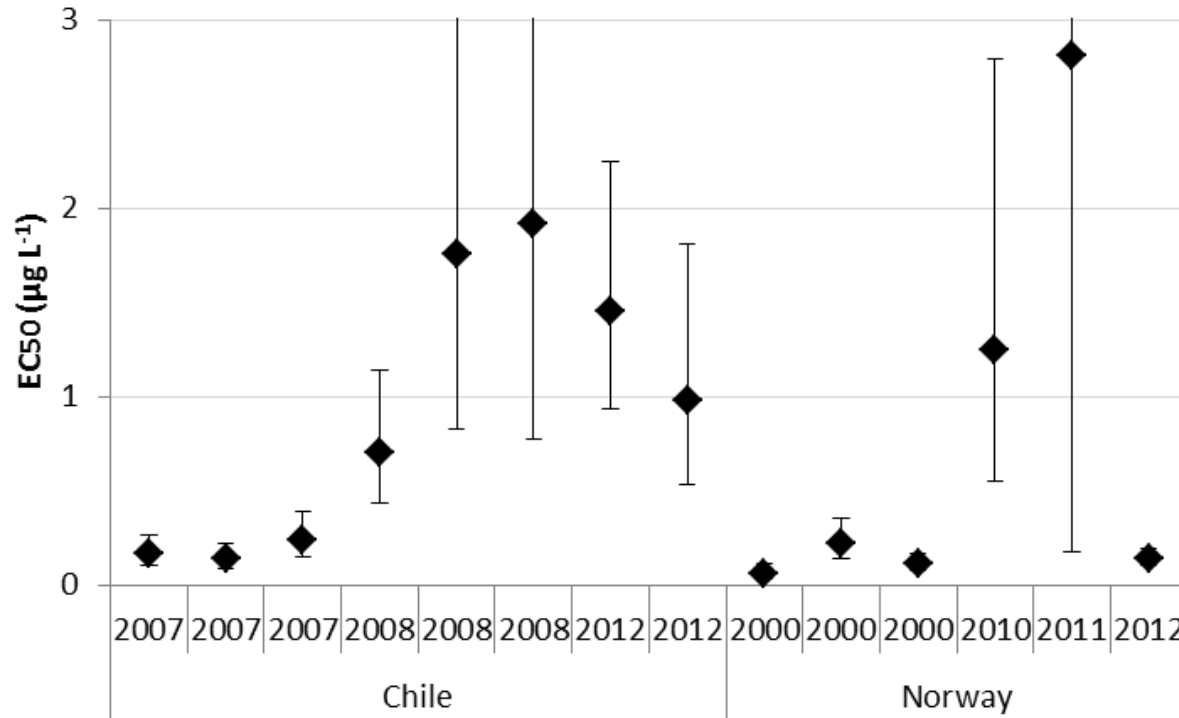
HRM-assay som skiller mellom homozygot følsomme (røde), heterozygote (blå) og homozygot resistente parasitter (grønne)

Full 5-dose bioassays – some results



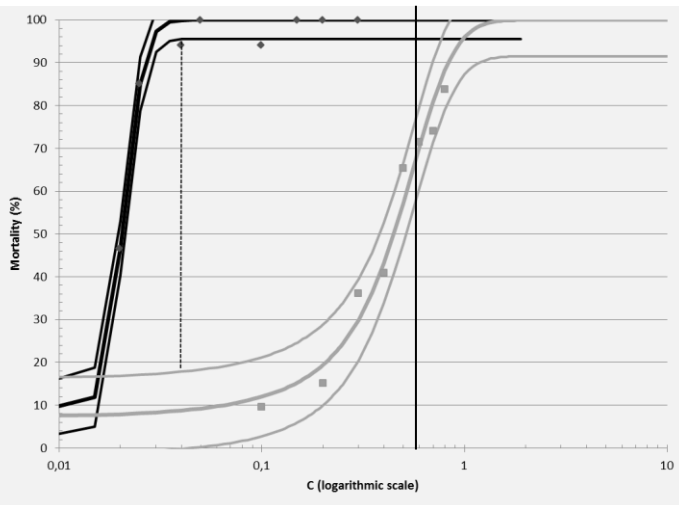
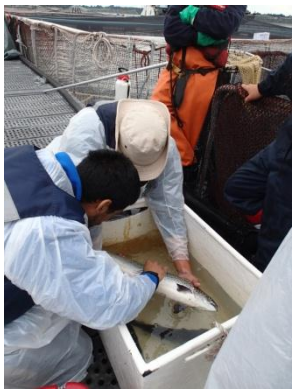
Sevatdal, unpublished

Pyrethroid resistance in Norway and Chile (5-dose assay)

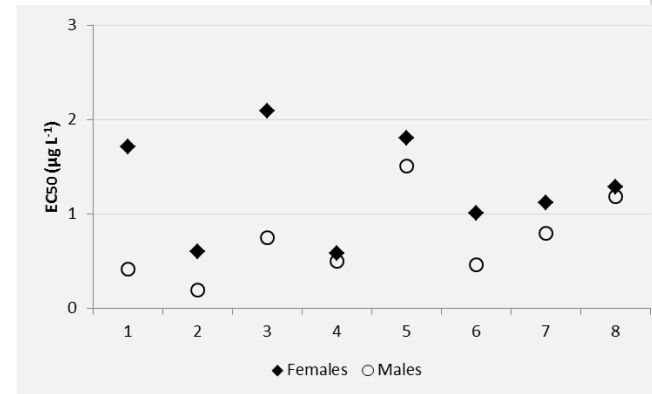
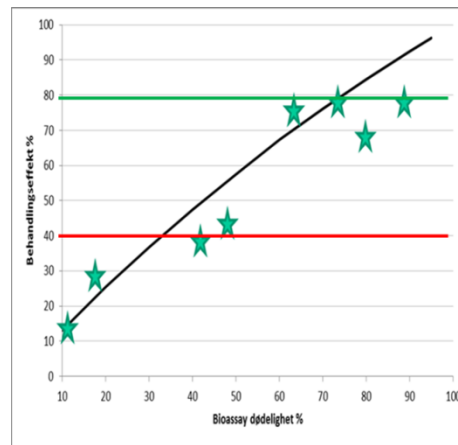


Helgesen et al., in press.

Results with simplified bioassays in Chile



Helgesen et al., 2013

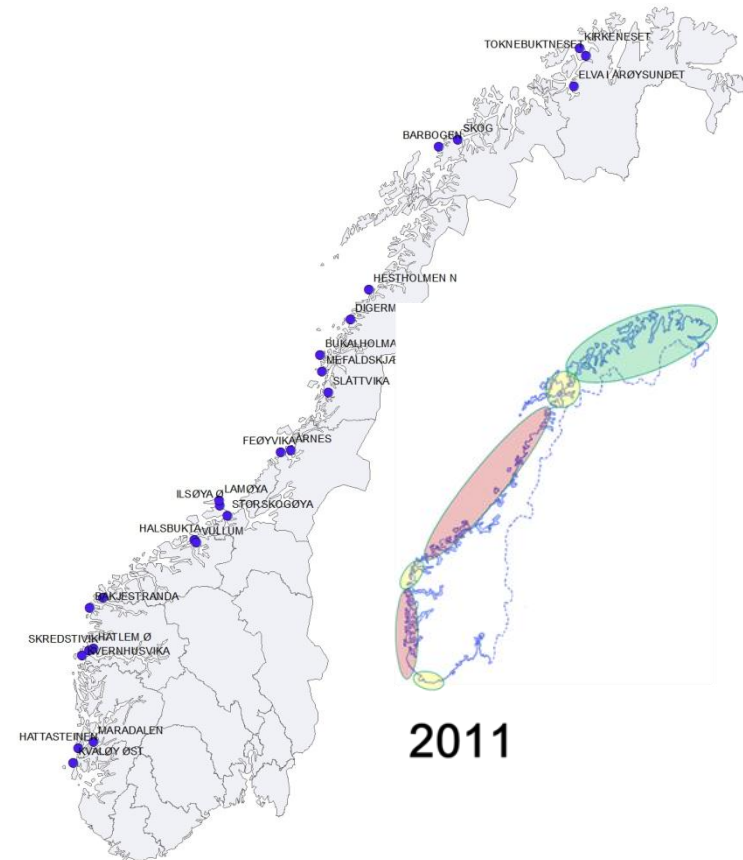


Helgesen et al., in press.



Resistance surveillance program in Norway 2013

- Initiated by the Norwegian Food Safety Authority
- Co-ordinated by the Veterinary Institute
- Based on 2-dose assays in 50 locations along the coast
- Testing sensitivity against azamethiphos, deltamethrin and emamectin benzoate

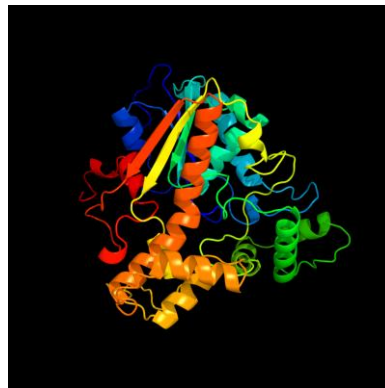


Biomarkers for resistance

- Pyrethroids
 - Biomarkers identified by Frank Nilsen's group in Bergen
- Organophosphates
 - Biomarkers identified by Tor Horsberg's group in Oslo
 - The markers are patented, but not yet published
 - Commercial PCR (TaqMan) assay developed by PatoGen AS – still undergoing validation
 - Available from 2014
- Biomarkers are mechanism-specific. If a new mechanism occurs, resistance will not be detected

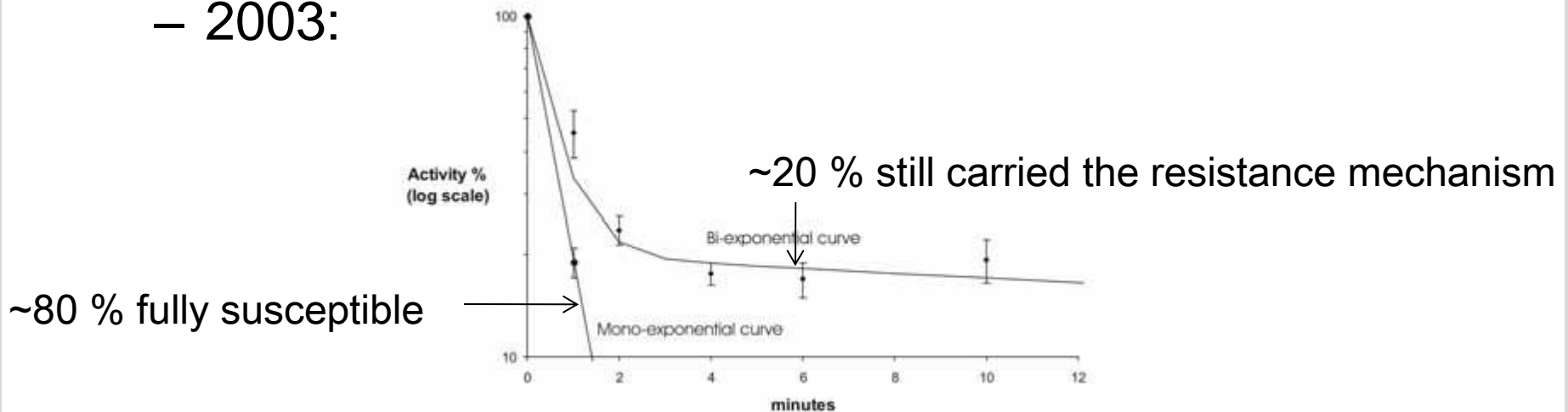
Can resistance disappear again?

- Resistance without a fitness cost
 - Likely to get established permanently
- Resistance mechanisms with a fitness cost
 - The frequency of resistance alleles will drop once the selection pressure (treatments) stops
 - They will not disappear completely from the population



Organophosphate resistance in Norway

- Organophosphates introduced in 1978
- Organophosphate resistance: 1991 => **13 years**
- The use of organophosphates was terminated in 1999
- 2003:



Fallang et al. 2004, Pest Manag Sci

- Organophosphates (SalmosanTM) reintroduced in 2008
- Very good initial efficacy
- Organophosphate resistance: 2009 => **1 year**

ROTATION OF TREATMENTS

Example of a rotation plan



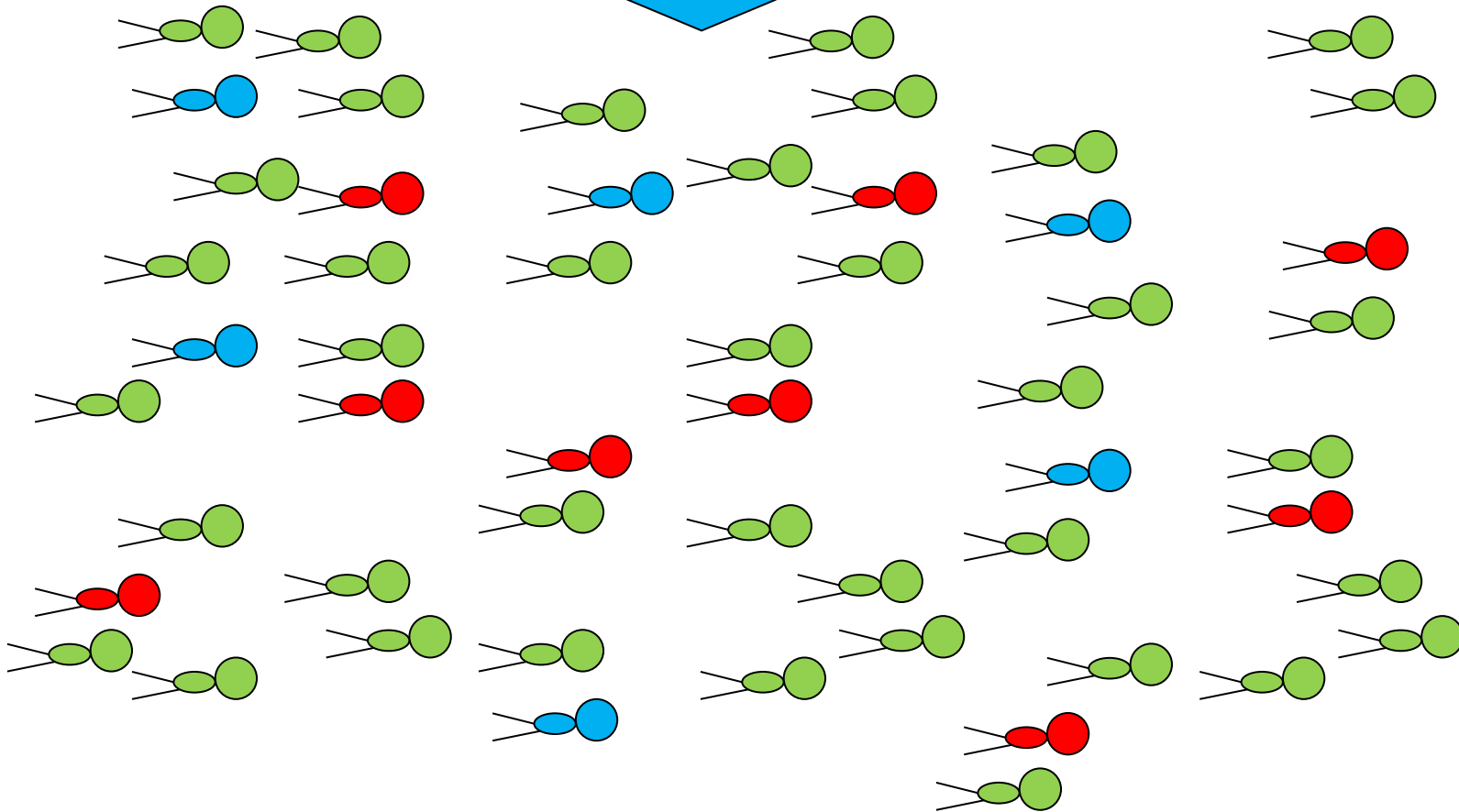
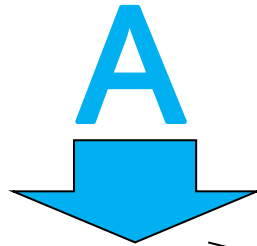
	SENSITIVE	EMAMECTIN RESISTANT	EMAMECTIN + PYRETHROID RESISTANT	EMAMECTIN + PYRETHROID + AZAMETHIPHOS RESISTANT
APR				
MAY				
JUN				
JUL	pyrethroids	pyrethroids	wrasses only (chitin inh.)	Area following No restocking
AUG		azamethiphos		
SEP				
OCT				
NOV	emamectin	hydrogen peroxide	hydrogen peroxide	hydrogen peroxide
DEC				
JAN				
FEB				
MAR	SPRING ACTION	SPRING ACTION	SPRING ACTION	SPRING ACTION
APR				
MAY				
JUN	pyrethroids	pyrethroids	hydrogen peroxide	hydrogen peroxide
JUL				
AUG			azamethiphos (chitin inh., combinations)	(chitin inh., combinations)
SEP				
OCT				
NOV	azamethiphos	azamethiphos		Premature slaughtering
DEC				

Can resistance be reversed by rotation of treatments?

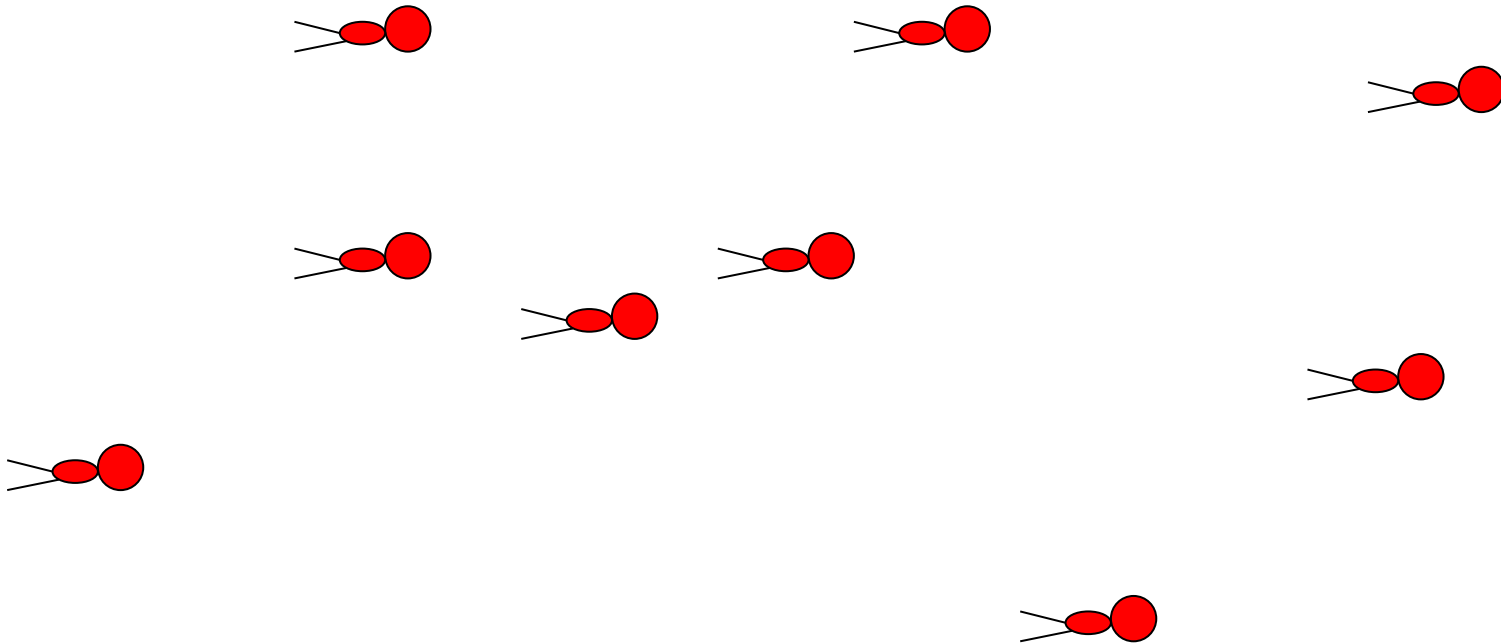
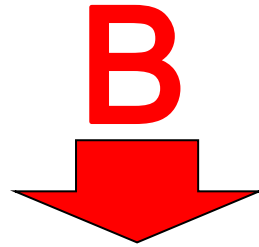


- A rotation plan can only work if there are two or more effective treatments available
- Reversal only seen when use of an agent is completely stopped for a loooong period of time
- No indication of reversal when the agent is used actively, however ...
- Indications that resistance development is slowed considerably down by rotation

What is accomplished by rotation?



What is accomplished by rotation?

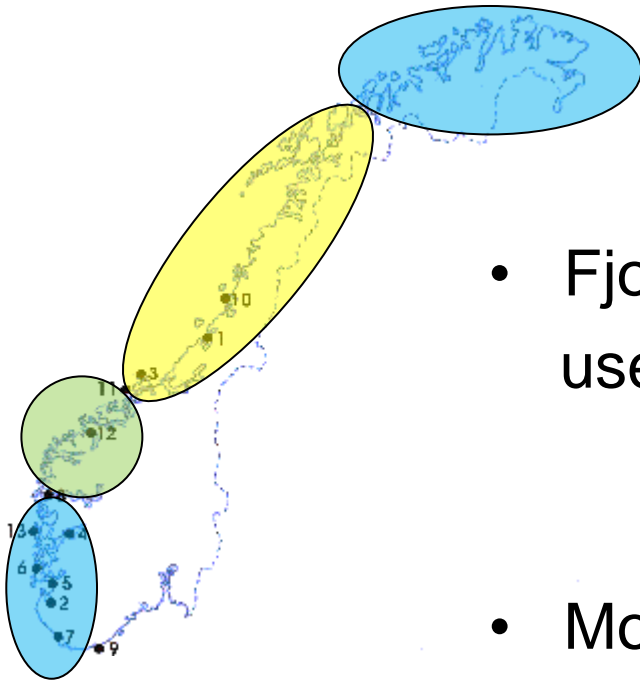


What is accomplished by rotation?

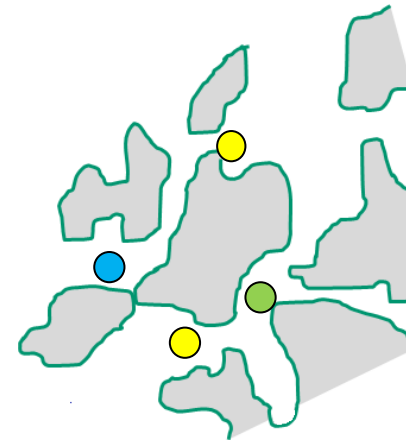


What is an optimal rotation?

- Area synchronisation of chemicals used?

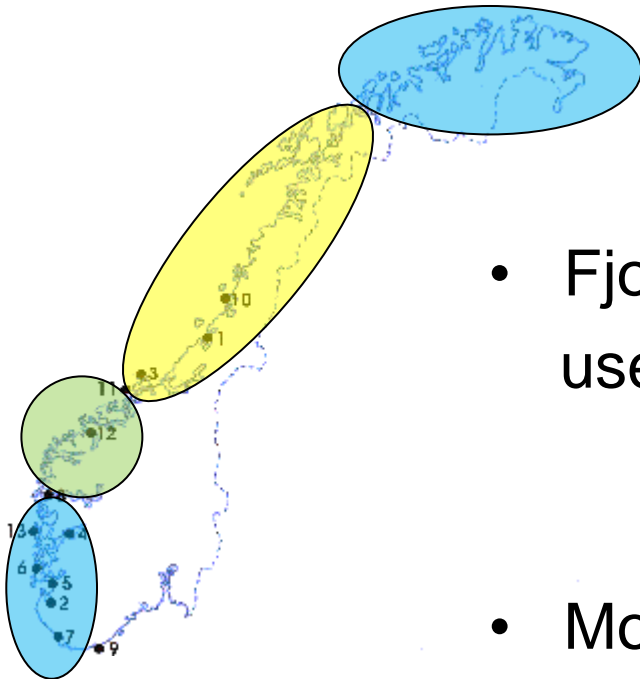


- Fjord/bay synchronisation of chemicals used?
- Mosaic treatments?

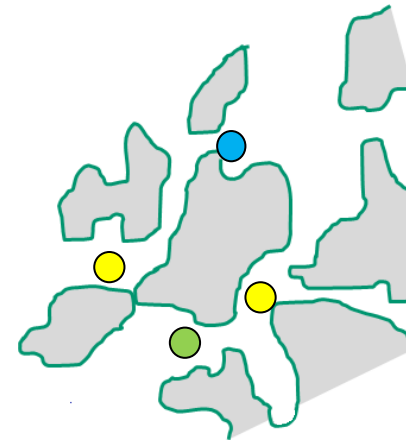


What is an optimal rotation?

- Area synchronisation of chemicals used?



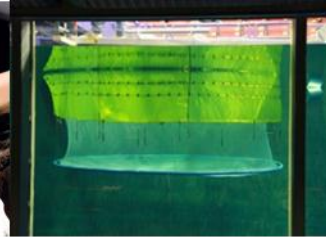
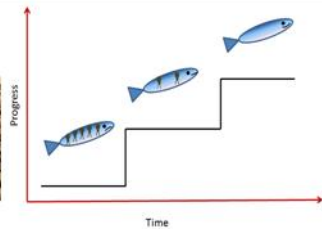
- Fjord/bay synchronisation of chemicals used?
- Mosaic treatments?



Non-medicinal control options



- A variety of non-chemical control options under development



PROTECTION OF SENSITIVE LICE



- The sensitive parasites are the easiest to kill
BUT
- When the last sensitive louse is killed, the battle is lost!
- Is it possible to keep the parasite numbers below 0.5 adult females per fish AND avoid resistance?



Can we rely on wild fish populations to preserve fully sensitive parasites?



- Western Canada:
 - The only treatment for 12 years has been emamectin benzoate (Slice™)
 - No resistance problems detected so far
 - Ratio wild salmonids : farmed salmonids = 1000 : 1

=> PROBABLY YES
- Norway:
 - Several treatments available (organophosphates, pyrethroids, emamectin benzoate, chitin synthesis inhibitors, hydrogen peroxide)
 - Significant resistance problems
 - Ratio wild salmonids : farmed salmonids = 1 : 1000

=> NO!

Are there other ways to preserve sensitive parasites?



- Revert to sub-optimal treatment methods?

=> No

- Acceptance of higher tolerance levels when non-chemical control options are used?

=> Acceptable in some cases?

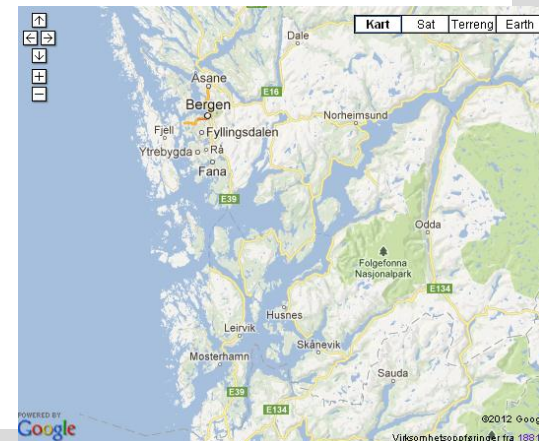
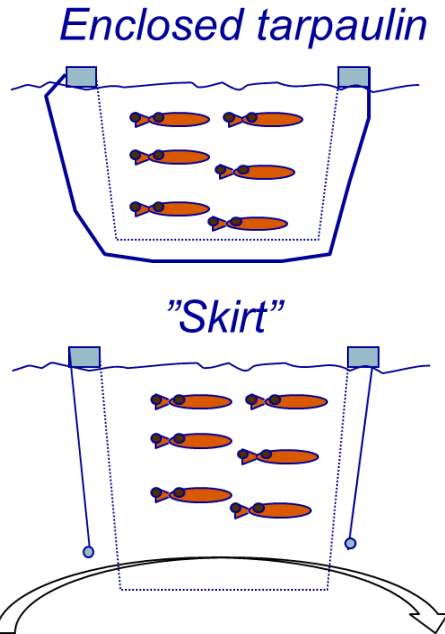
- Non-treated cages?

- Australian regulations state that only 90 % of cotton fields can be sprayed, 10 % must be left alone

=> Acceptable in some cases?

- Non-treated areas, e.g. areas with brackish water?

=> Acceptable?



Without a proper plan for conservation of sensitive parasites, there is little hope to fight resistance!



thanks