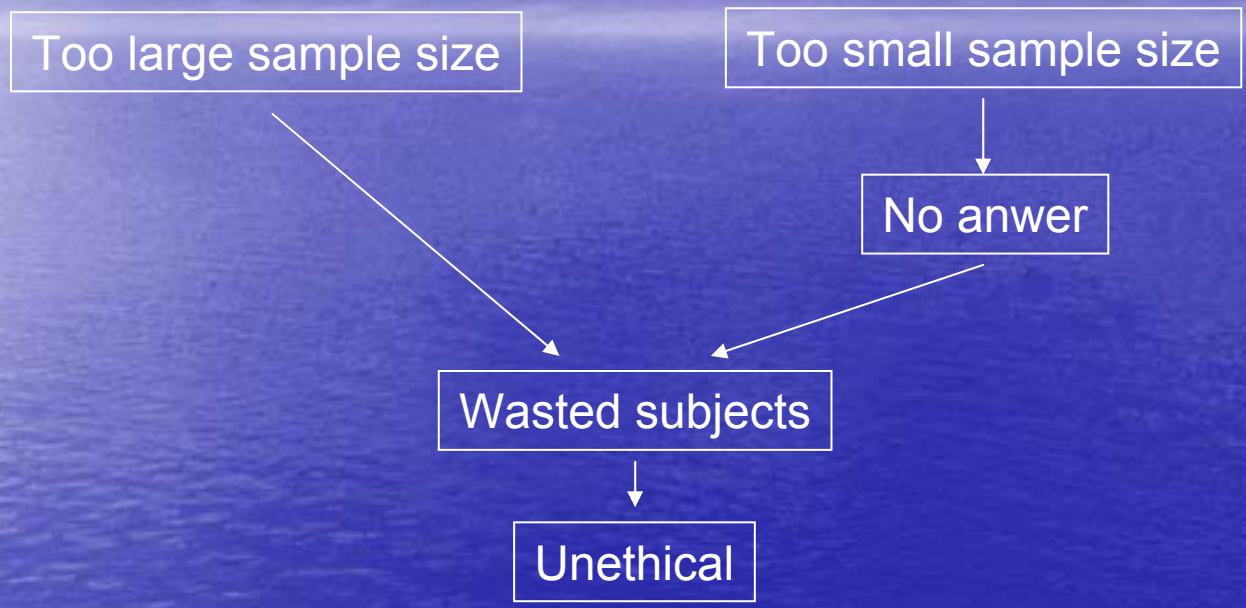
Choosing the correct number of fish in laboratory and field experiments

Stig Larsen (Prof. Contr. Clin. Trials)
Norwegian School of Veterinary Science

Unethical situation



- The necessary number of subjects to be included in a study has to be calculated before start of trial

Factors influencing the sample size

- The probability of erroneously claimimg difference between groups (Type I errors or significance level). {Wanted as small as possible}
- The probability of detecting a real difference between groups. (Detection level or Type II error). {Wanted as large as possible}
- Clinical relevant difference. How large should a difference be in order to be of clinical interest?
- Optimalisation of study design.
- Degree of heterogeneity in the study population.
- Observation methodology.

Specific influence of the factors

- Significance level [$p \leq \alpha$]
  - The probability of erroneously concluding changes whithin or differences between groups.
- Detection level β [Type II error = $1 - \beta$]
  - The probability to detect an actual change whithin or differences between groups.
- Clinical relevant difference [$\Delta = A \times \sigma$]
  - The minimum change within or difference between group which is of clinical relevance to detect.

Patients as own control

- Sample size for different values of the significance level α, the detection level β and the clinical relevant difference Aσ

A	1.00	0.95	0.90	0.85	0.80	0.75	0.70
α=0.05 β=0.95	12	16	16	16	20	24	24
α=0.05 β=0.95	20	20	24	28	32	36	36
α=0.05 β=0.95	20	20	24	28	28	32	36
α=0.05 β=0.95	24	28	32	36	40	44	52

Comparison of groups

- The number of subjects to be included in each group in the parallel group designed trial related to different values of the significant level, the detection level and the clinically relevant difference.

Significance A1level	Detection level	Clinically relevant difference = Dτ						
		τ 10cm	0.95τ 9.5cm	0.9τ 9cm	0.85τ 8.5cm	0.8τ 8cm	0.75τ 7.5cm	0.7τ 7cm
α=0.05	β=0.90	18	20	23	25	29	32	37
	β=0.95	23	26	28	32	36	41	47
	β=0.99	34	37	41	46	52	59	68
α=0.025	β=0.90	22	25	28	31	35	40	45
	β=0.95	28	31	34	38	43	49	56
	β=0.99	39	43	48	54	61	69	79
α=0.01	β=0.90	28	31	34	38	43	49	56
	β=0.95	34	34	41	46	52	59	68
	β=0.99	46	57	57	63	72	81	93

Comparison of design

Table: α=0.05 β=0.95

Type of design	Clinical relevant difference (A) 1	0.9	0.8	0.7
Cross-over design	12	16	20	24
Two-group design	46	54	70	92

The influence of observation methodology

- Binomial observation
- Multinomial observation

- Discrete variable

- Continuous observation - Continuous var.

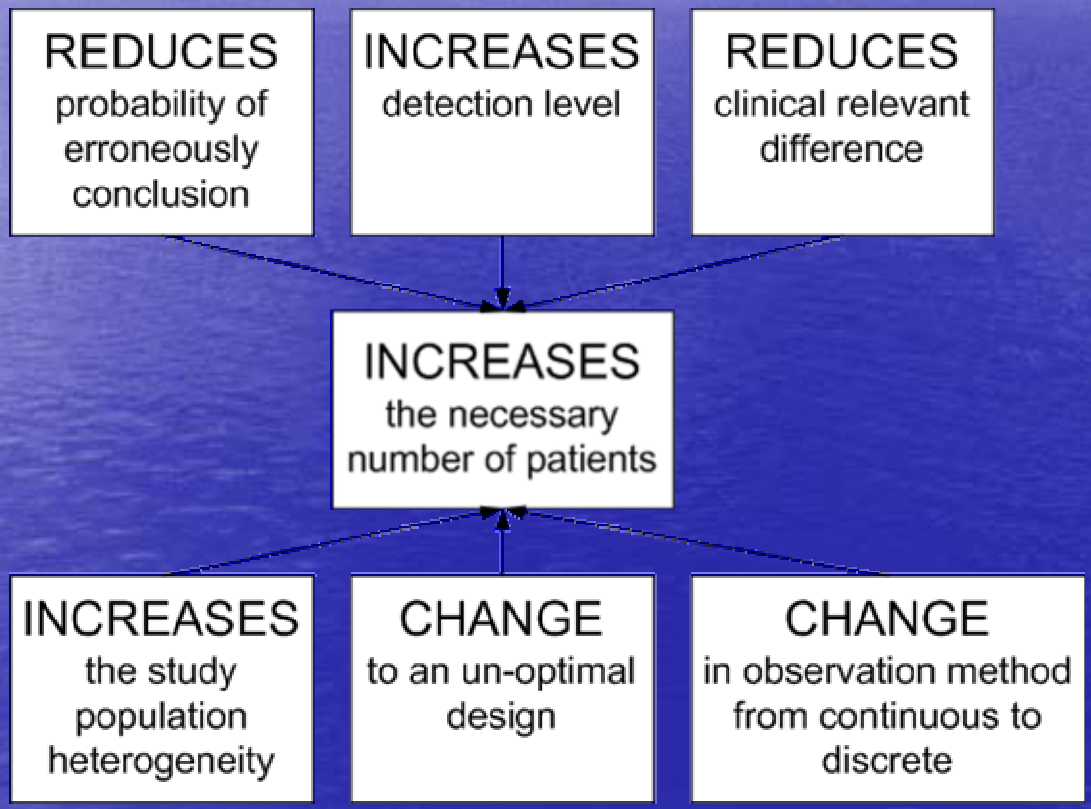
Binomial situation

- Occurence of event/symptom
  - Fatal/not fatal outcome
  - Healed/not healed outcome

Sample size in the binomial situation

	Clinical relevant difference (A)		
	10%	**20%**	**30%**
α = 0.05 β = 0.95	1068	256	106
α = 0.05 β = 0.99	1556	370	152
α = 0.10 β = 0.95	844	202	82
α = 0.10 β = 0.99	1282	304	124

Summary


REDUCES
probability of erroneously conclusion
INCREASES
detection level
REDUCES
clinical relevant difference
INCREASES
the necessary number of patients
INCREASES
the study population heterogeneity
CHANGE
to an un-optimal design
CHANGE
in observation method from continuous to discrete

Design overview

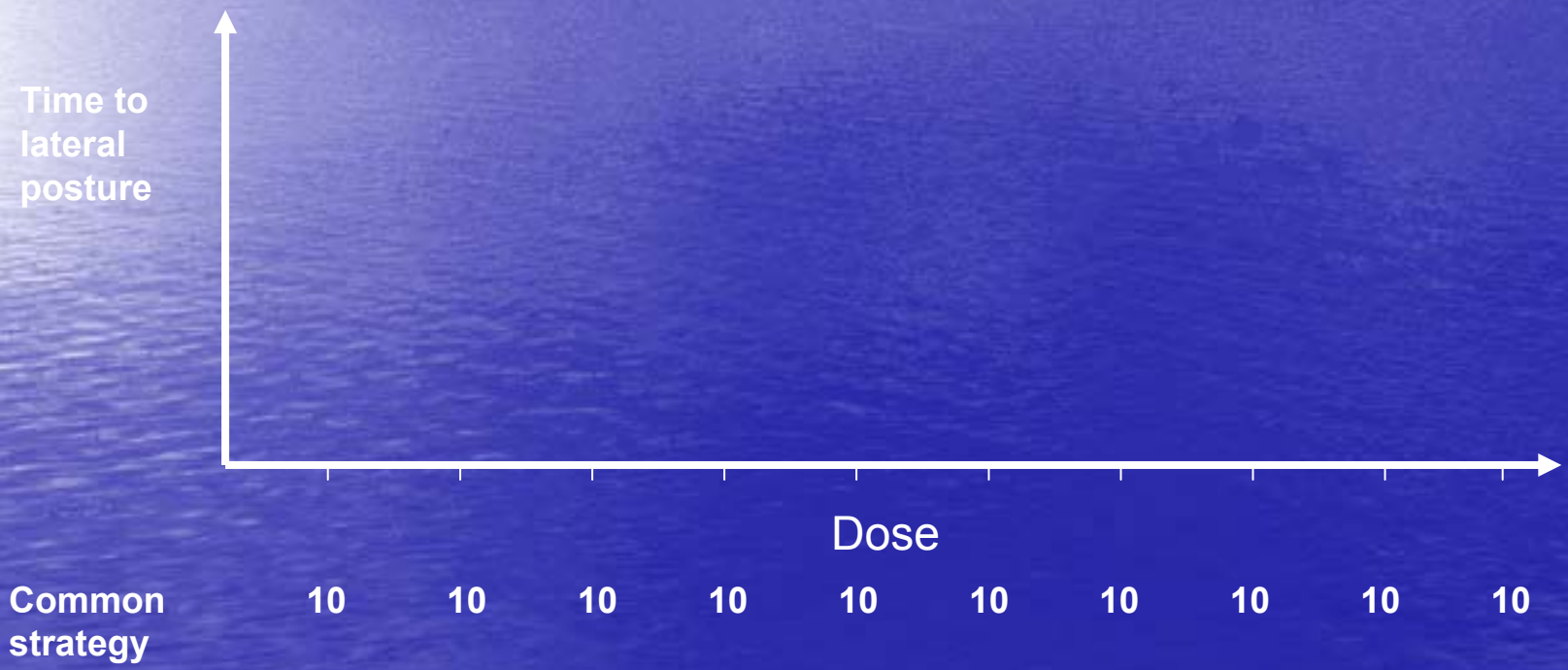
- Parallell group design
  - Two group design
  - Multiple group design
- Stratified design / Block design
- Factorial design
- Latin square design
  - Cross-over design
  - Semi-cross over design
  - Greako latin square design
- Multi cross-over design
- Adaptive design
  - Play-The-Winner (PTW)
  - Modified Play-The-Winner (MPTW)
  - Randomizied Play-The-Winner (RPTW)
  - Weighted Play-The-Winner (WPTW)
- Sequential design
- Response suface design

Responce surface design

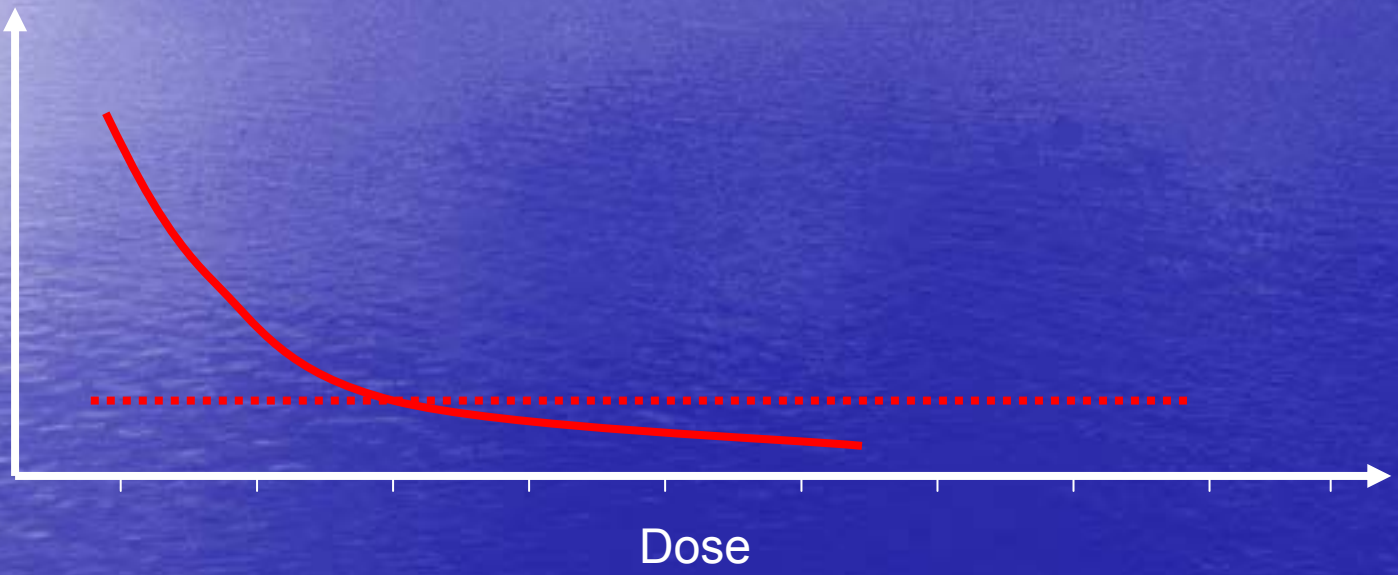
- Aim: Dose finding studies

- Background situation
  - With some *a priori* knowledge
  - Without or limited knowledge

Dose-finding studies
Example: Immobilization of fish

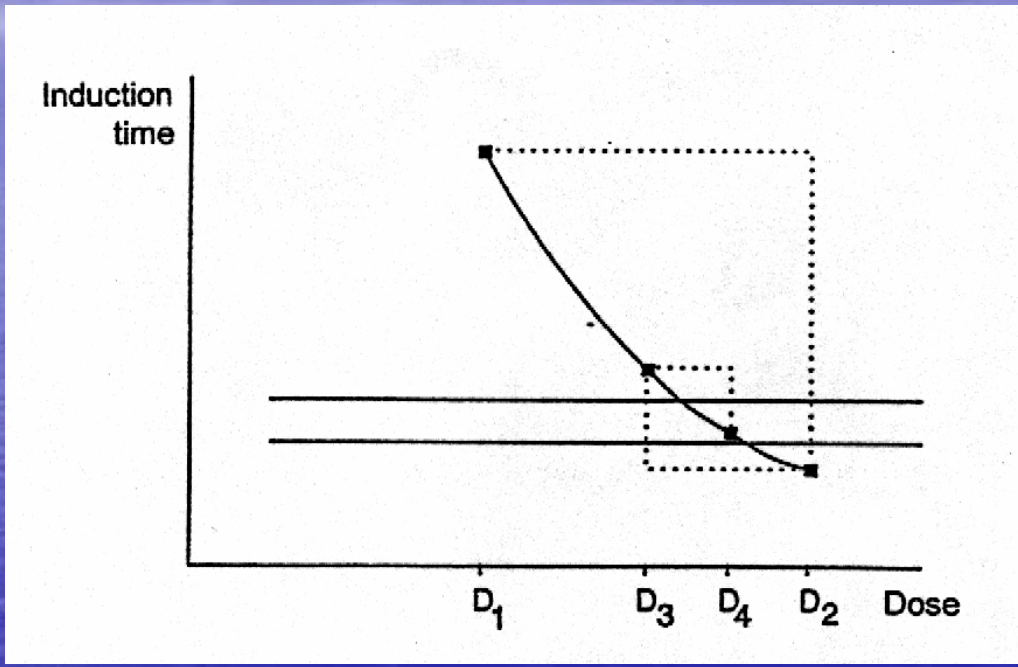


Dose-finding studies
Example: Immobilization of fish



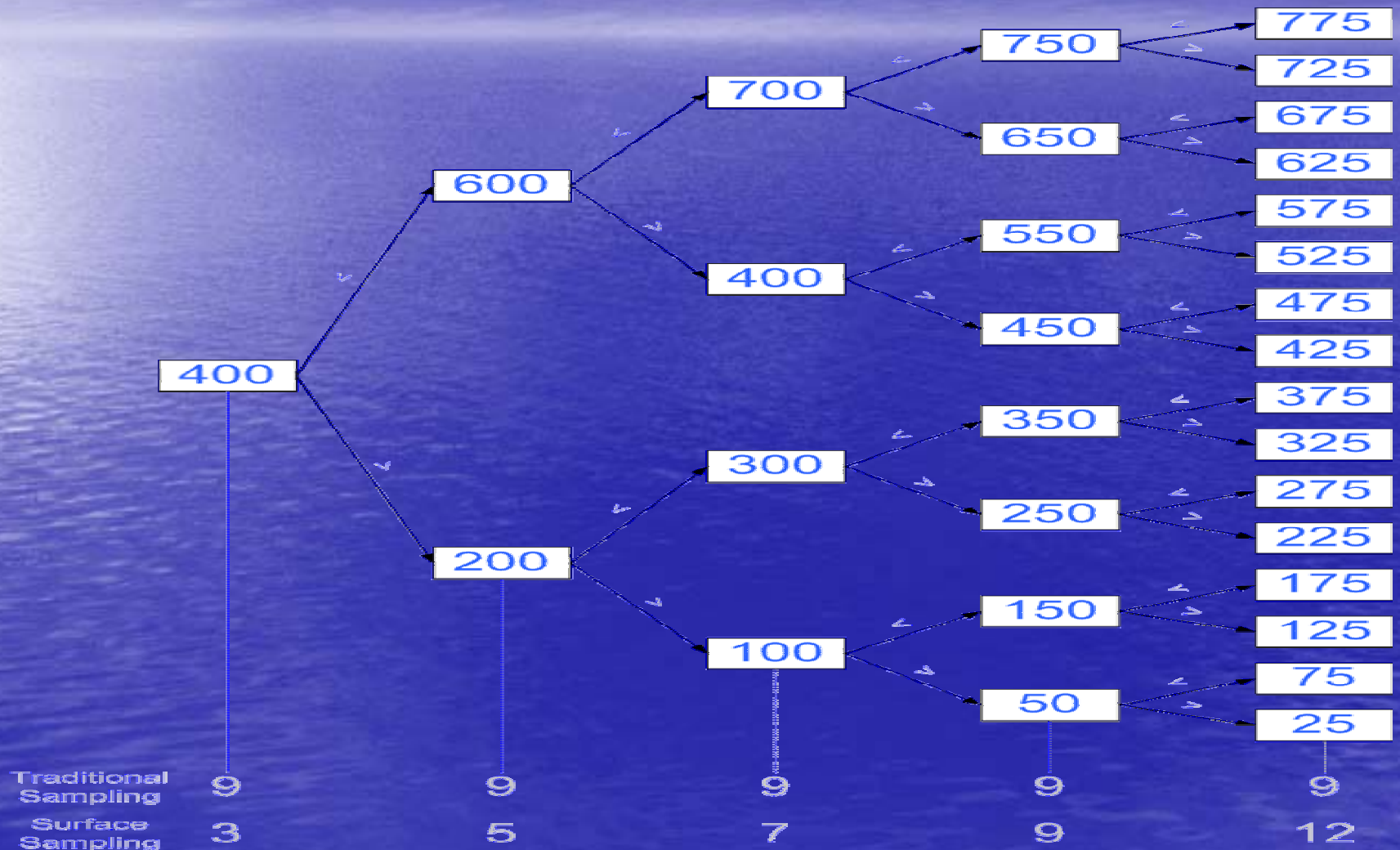
Common Strategy	10	10	10	10	10	10	10	10	10	10 = 100
Possible response surface design	1	7	12	7		1				= 51
		5		9	9					

Response surface design with iteration



- In order to get similar power as obtained by 16 fish in this design, 198 fish have to be included in a traditional design.

Response surface design with pathway procedure



Conclusion

- In order to reduce the number of fish used in both field and laboratory experiments, it is crucial to:
  - Choose an optimal study design
  - Optimize the information from each included fish