Research: Philosophy, Methodology and Pitfalls

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Outline

1. What is research and what is GOOD research?
2. Different types of research
3. Key processes in conducting research
4. General principles and common pitfalls in experimental design and data interpretation


Now, rebuttal from students, Please!
Teacher
SCHOOL

Quiz:
What is “philosophy”?

Philosophy

"*Study of the truths and principles of the universe, life, and morals, and of human understanding of these*"
(Oxford dictionary)

Quiz:
What is research?

What is research?

1. Collection of data
2. Analysis of data
3. Interpretation of data

What is research?

- Is the conduct of polling survey "research"?

Quiz:
What is GOOD research?

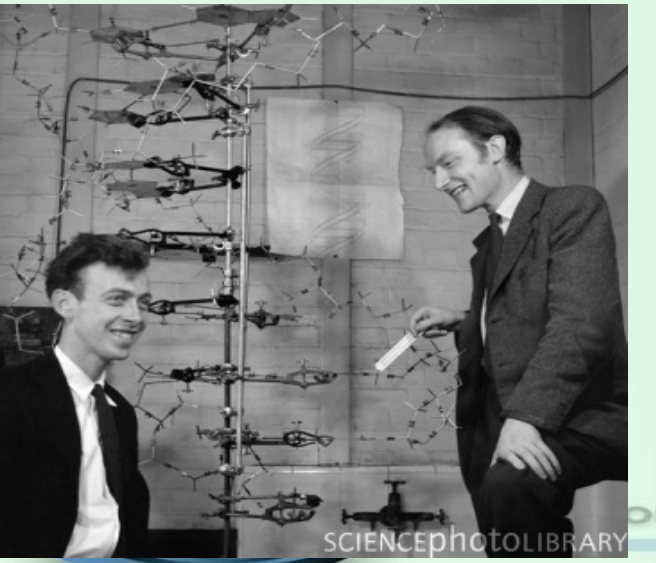
Good research: Novelty and Originality

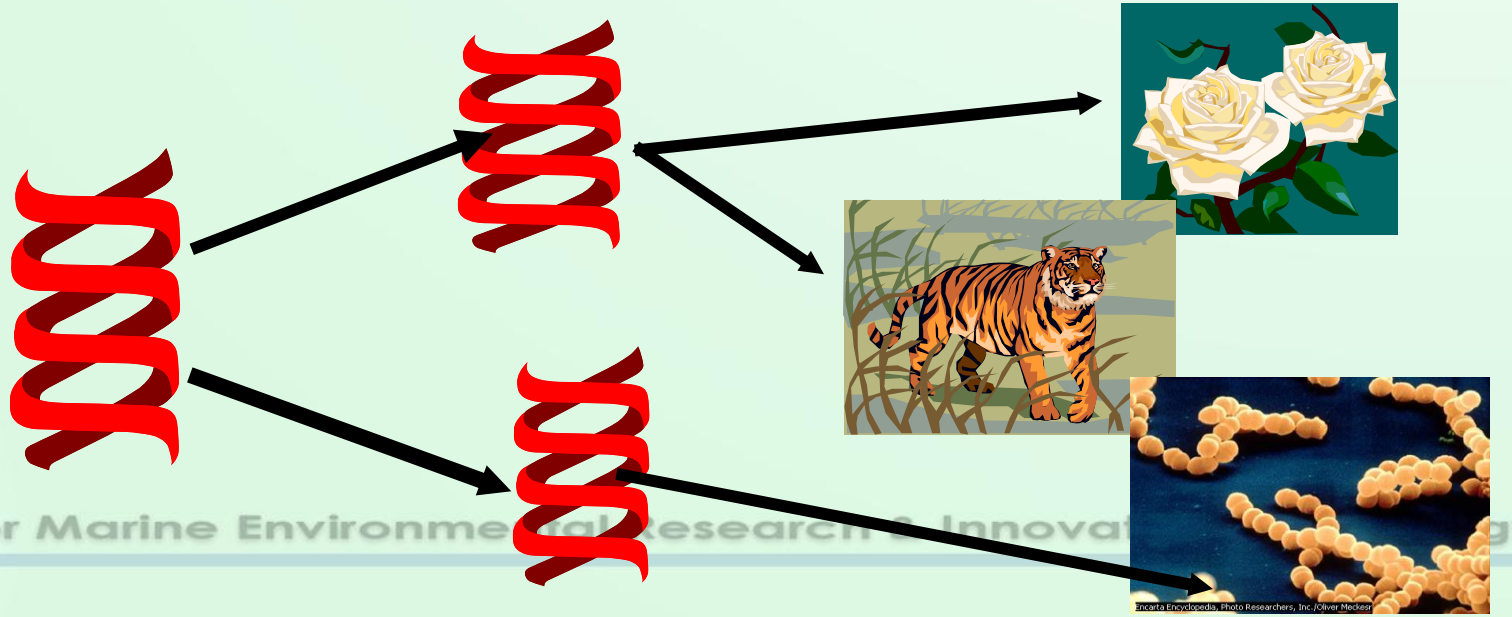
- Never done before
- Advance our knowledge and understanding

  Enough? Anything else?

Good research: Generalization

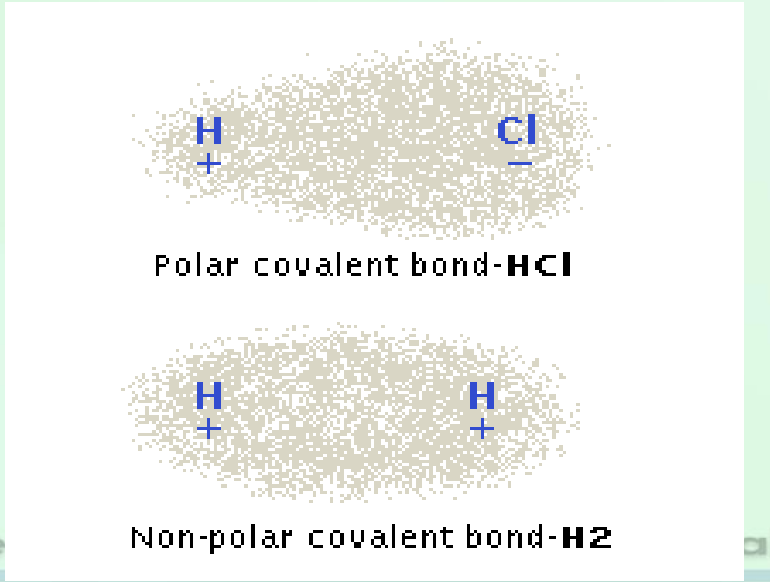
- DNA structure (James Watson & Francis Crick, Nobel Laureate 1962)
  - Genetic code for all living organisms
  - Explain replication and protein synthesis





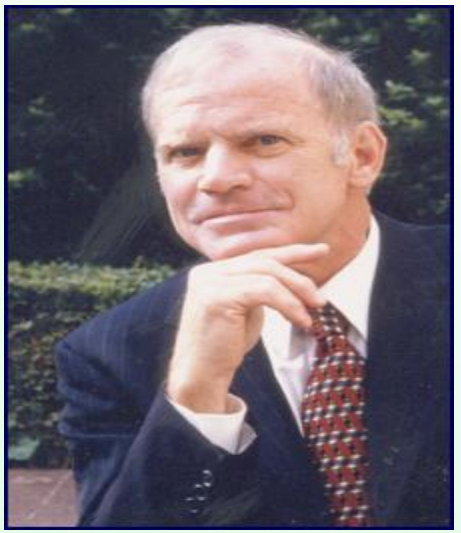
Good research: Prediction

- Chemical bonding and forces between molecules (Linus Pauling, Chemistry Nobel Laureate 1954)
  - Predict molecular interactions and chemical reactions



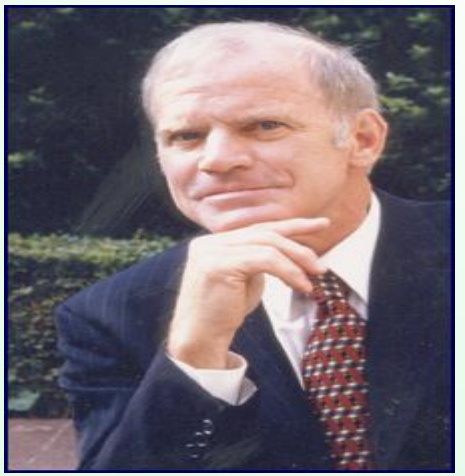
Good research: Wide application

- Polymerase Chain Reaction (Kary Mullis, Nobel Laureate in chemistry 1993)
  - Make 1 million copies of DNA within hours
  - Wide application in medicine, forensic sciences, molecular biology, genetics, biotechnology
  - Form the basis of paleobiology

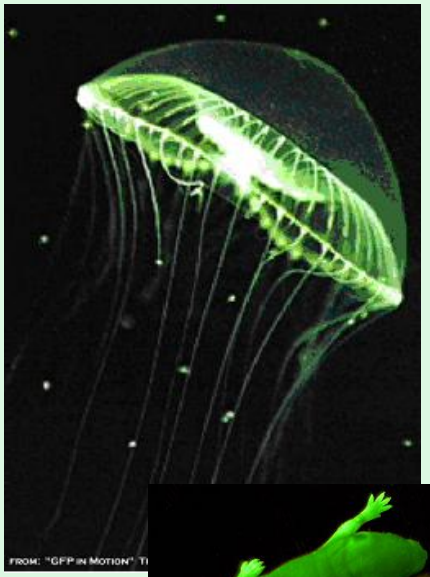


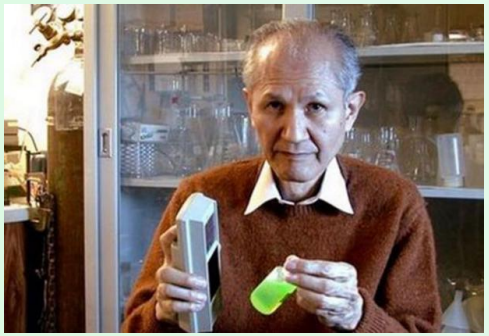
Good research: Wide application

- Polymerase Chain Reaction (Kary Mullis, Nobel Laureate in chemistry 1993)
- **Number of publications = 23**
- **Times cited = 27,930**
- **H-index = 16**



Good research: Wide application





Osamu Shimomura Martin Chalfie Roger Y. Tsien

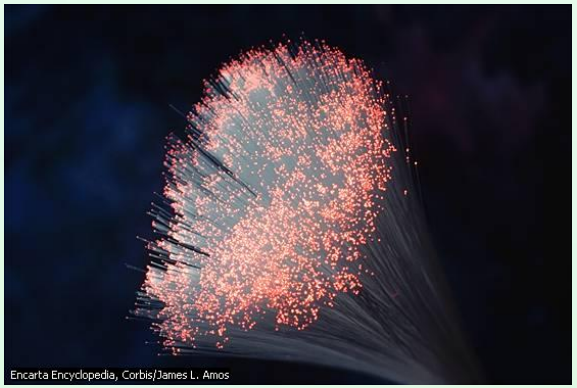
Nobel Laureate 2008

Green Fluorescent Protein (GFP)

Enable us to watch expression of a specific gene, development of cells, spreading of cancer cells etc.

Good research: Wide application

- Fiber optics (Charles Kao, Nobel Laureate 2009)
  - Light loss in glass fiber due to scattering and absorption of impurities
  - Lead to the development of silica fiber of sufficient purity to carry IR for > 100 km, speeding up transmission of signals and lowering energy requirements



Good research: Wide application

- Microsoft Windows
- Search engines (e.g. Google)

Vital elements of good research

- Originality & Novelty
- Able to make generalization
- Able to make prediction
- Wide application

Ask yourself this question:

"*How about my own research project*?"

Two useful questions for your self reflection

- So What?
- Who cares?

Types of Research

Types of Research

1. Fishing expedition
2. Discovery
3. Technique development
4. Hypothesis testing

Types of research

1. Fishing expedition (No specific idea, just try it out)
   - How many species of fish are there in Tolo Harbor?

Types of research

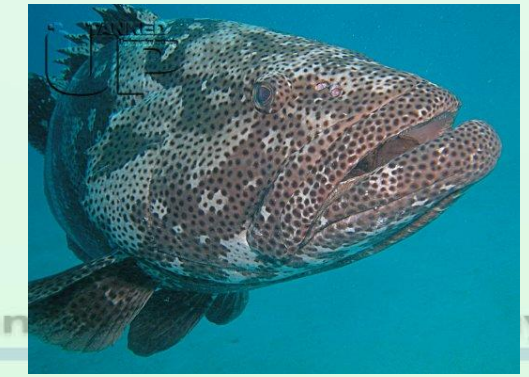
1. Fishing expedition (No specific idea, try it out)

   What is the level of cadmium in the sediment along the China coast?

Types of research

2. Discovery (Fact finding)

- Which species of fish is most sensitive to cadmium?
- What is the optimal temperature and pH for crystalizing compound A?



Types of research

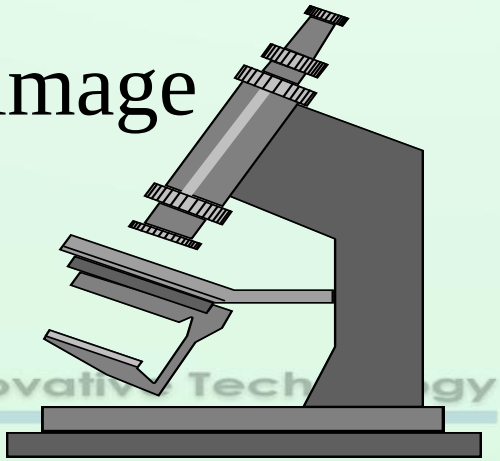
2. Discovery (Fact finding)

- What are the no. and proportion of yellow hair and white hair on MY dog?

Types of research

3. Technique development

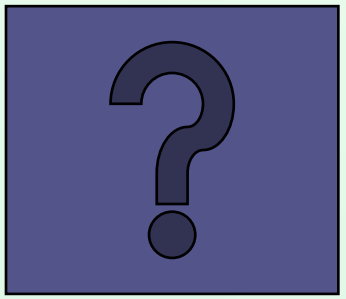
- Lower the detection limit of chemicals (from ppm to ppt)
- New/more user friendly program for faster calculation
- Improve the resolution of microscope / image recognition/telescope



Types of research

4. Hypothesis testing

- Can Drug A increase blood pressure?
- Can Chemical A increase the reaction rate ?

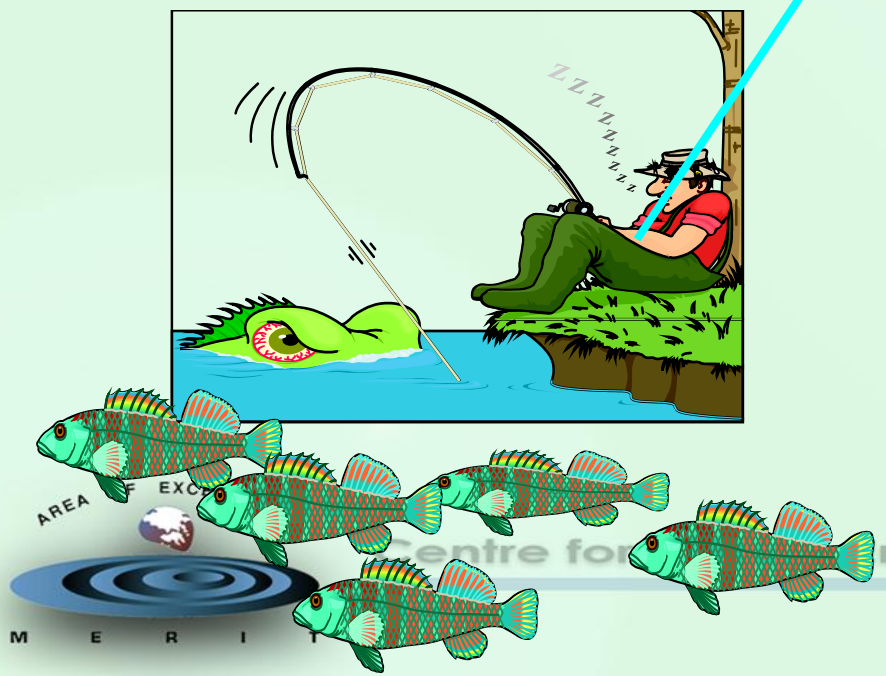


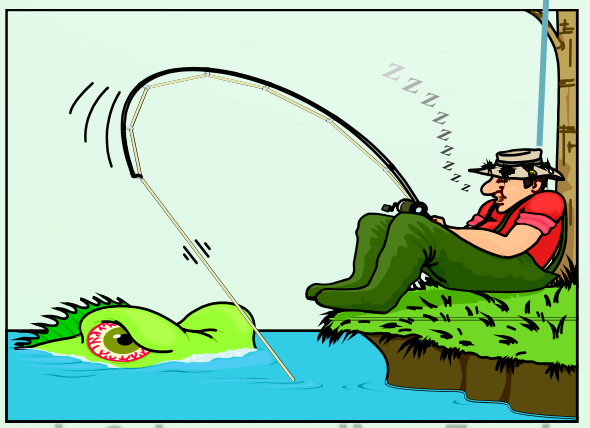
IMPORTANT: What makes you think that Drug A can increase blood pressure?

Tell the difference !

I'll go fishing Sunday 4 pm. I hope there will be a lot of fish

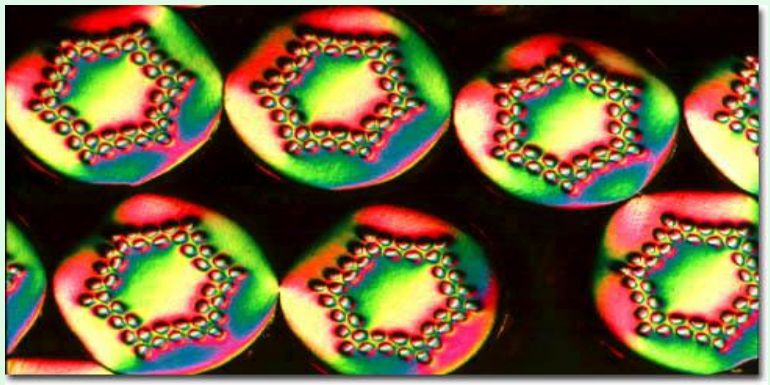
I'll go fishing 4 pm Sunday, because at that time the water temperature should be 20°C, tidal current should be minimal, and Oct. should be the spawning season of groupers





Discovery research can also have very significant impact

- Penicillin (Alexander Fleming, Howard Florey & EB Chain, Nobel Laureates, 1945)
- Superconductor (Heike Onnes, observed no electrical resistance in mercury below 4.2 K)

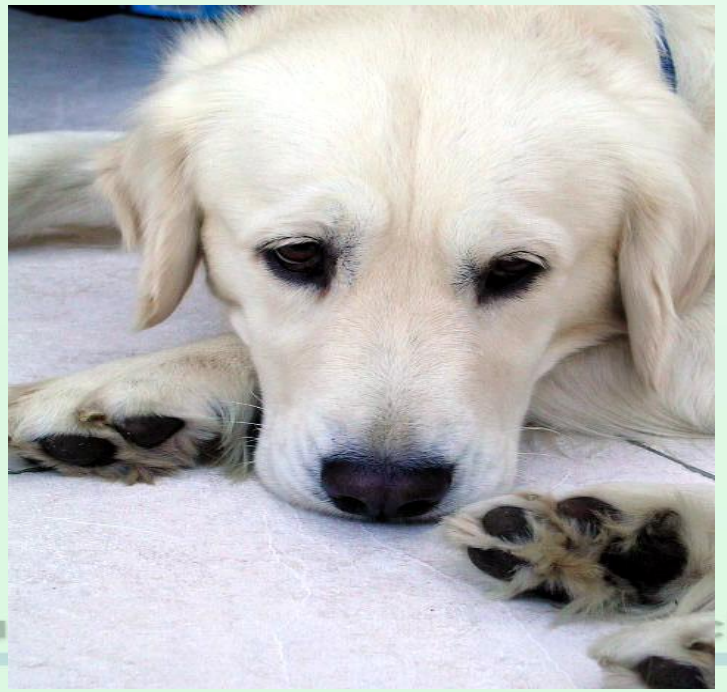


Types of research

1. Discovery (Fact finding)
   - What are the no. and proportion of yellow hair and white hair on MY dog?

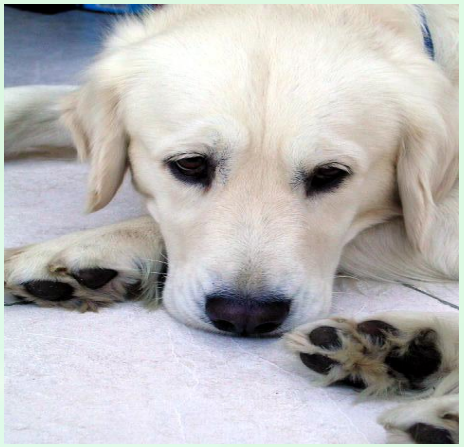
It would be entirely different and becomes **GOOD** research if:

- There are good indications that white hair and yellow hair on you dog and your neighbors' dogs all appear in certain proportion (e.g. 3 White: 1 Yellow)

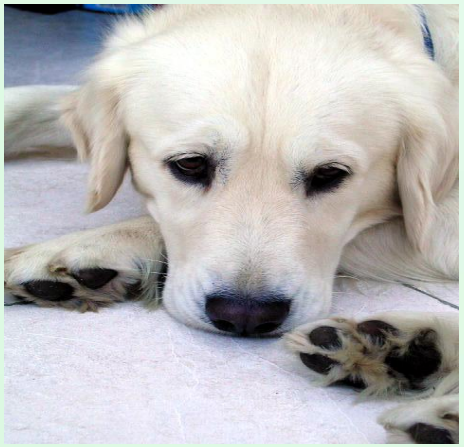


It becomes good research because

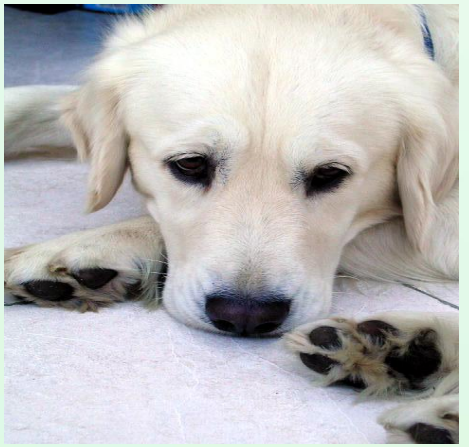
- Once you have verified this fact, you may make generalization and prediction on other dogs in HK, China or even better, worldwide



Hong Kong



China

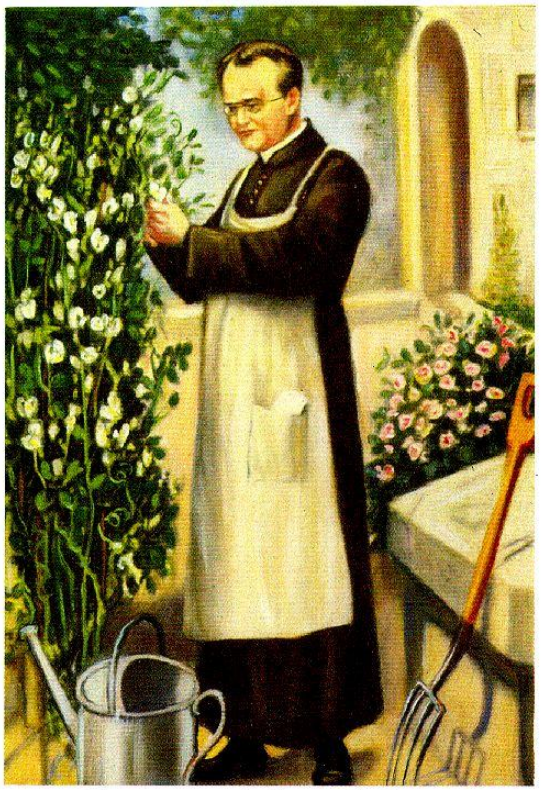


Worldwide

It would be EXCELLENT research if you further ask the question:

- Why is this 3:1 ratio found in all dogs?

  Remember how Mendel did his experiment on pea and come up with the 9:3:3:1 ratio in genetics?

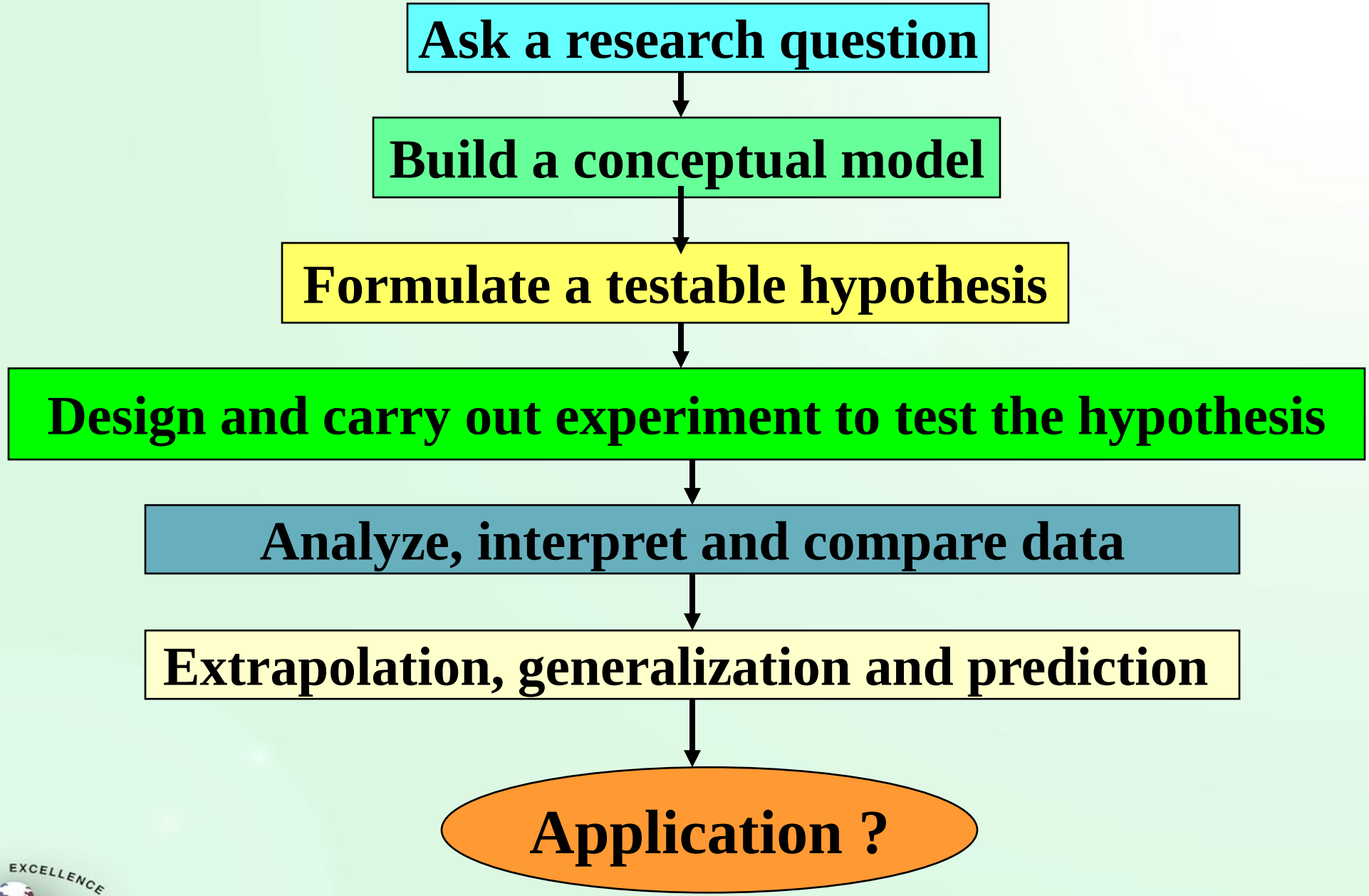


Gregor Mendel

How about your own research project?

- Fishing expedition?
- Fact finding?
- Technique development?
- Hypothesis testing?

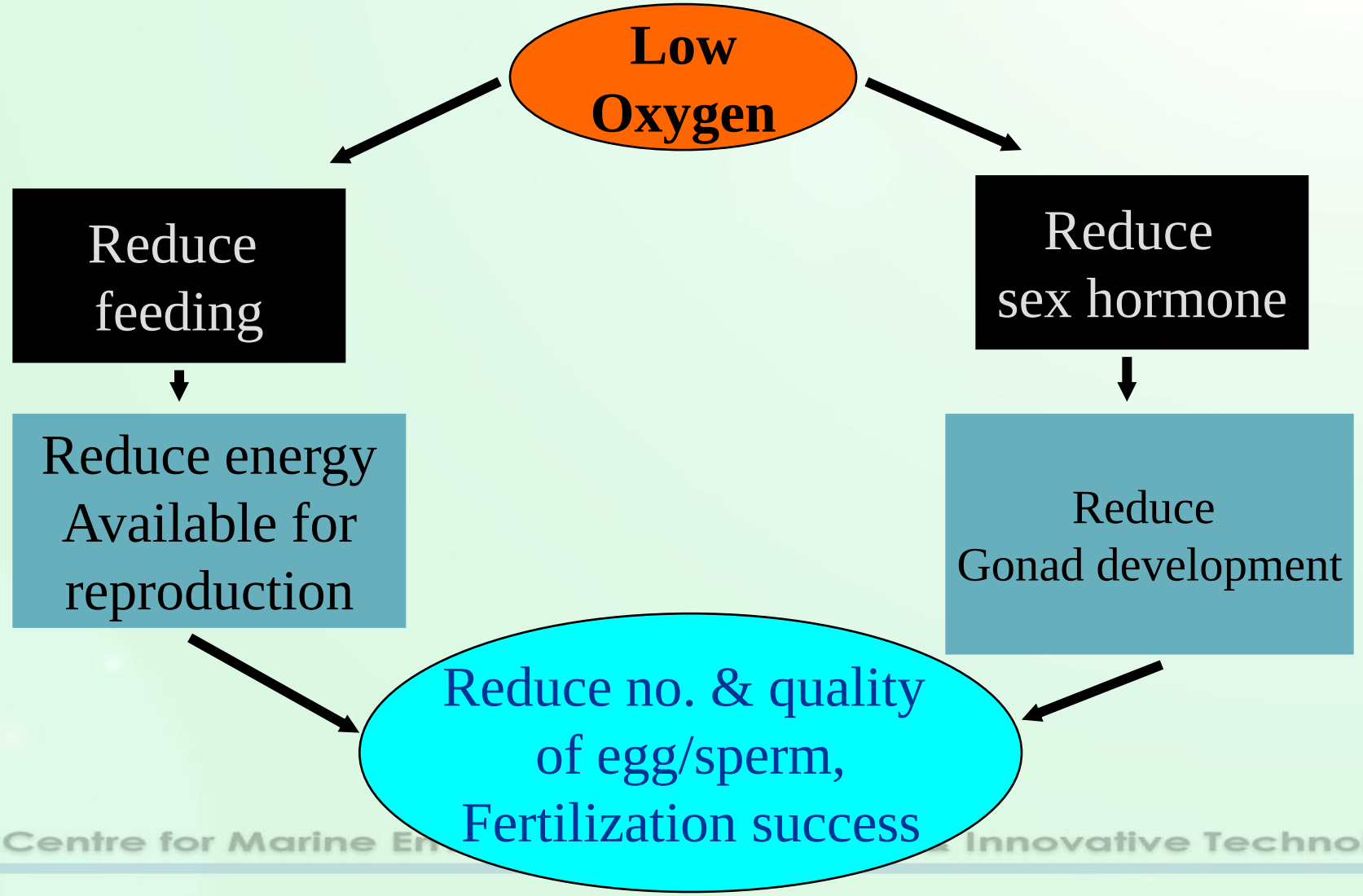
Key Processes in Research


Ask a research question
Build a conceptual model
Formulate a testable hypothesis
Design and carry out experiment to test the hypothesis
Analyze, interpret and compare data
Extrapolation, generalization and prediction
Application ?

1. Ask a Question

- Is reproductive output of fish lower when oxygen level is low?

2. Build a Conceptual model



3. Formulate testable hypotheses

1. Low oxygen reduces feeding?
2. Low oxygen reduces level of sex hormones (testosterone and/or estradiol or gonadotropins)?
3. Low oxygen reduces energy channeled to reproduction (A smaller gonad)?
4. Low oxygen affects gonad development (less mature sperm and eggs)?
5. Low oxygen reduces no. of eggs and sperm, gamete quality (sperm motility, size of egg) and fertilization success?

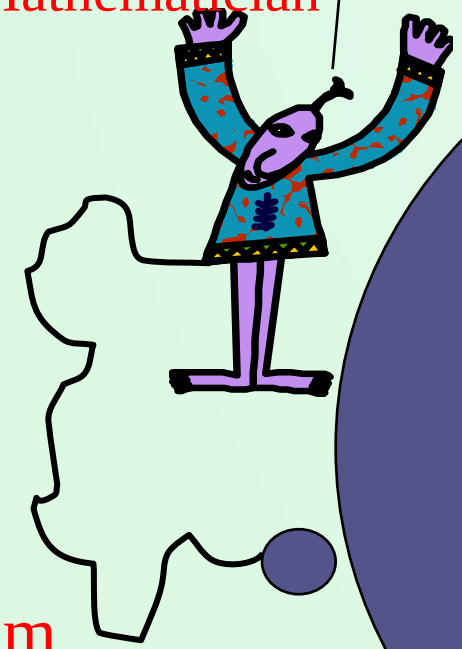
4. Design experiment to collect data and test you hypothesis

Hypothesis testing

- In statistics, nothing is ever "proved"
- Hypothesis is only rejected as "unlikely", and their logical counterpart is therefore accepted

It is impossible!
I give up!

Mathematician



4 m

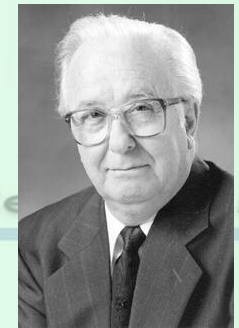
5 m

If I try for 1 million times, I may be able to get there

Statistician

4 m

In memory of Prof. Peter Larkin



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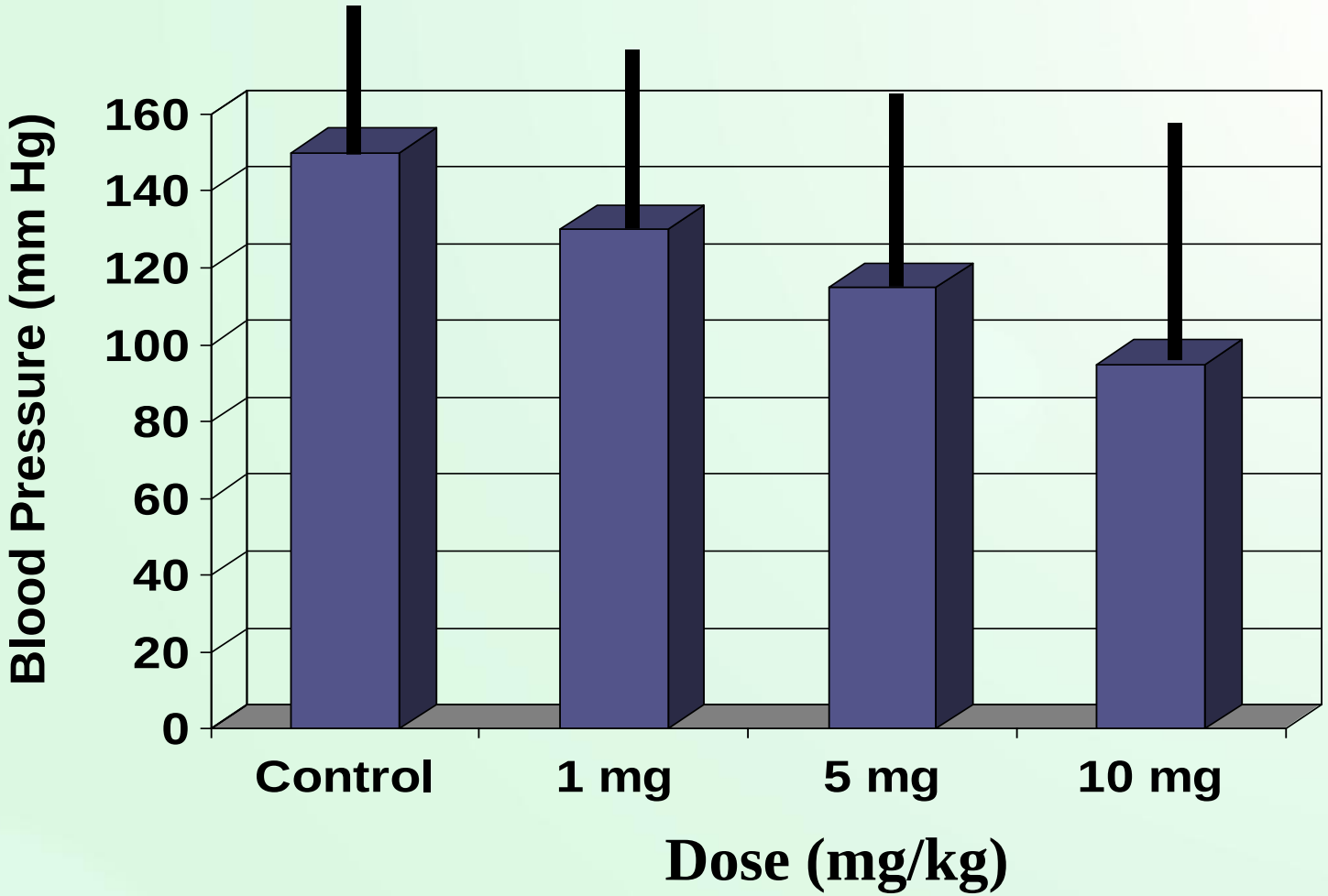
M E R I T

Ce Marine Environmental Research & Innovative Technology

Hypothesis testing

- Null hypothesis (Ho): **x =/=y**
- Alternative hypothesis (H_1) **x =y**
- Specify a probability level (α) that you are prepared to accept (e.g. **0.05, 0.01**) (accept only 5 time in one hundred that your results were due to chance)
- Do your experiment
- Perform appropriate statistics test on your experiment data (Calculate probability)
- If ***p* < α: reject Ho** (because it is unlikely) :accept H_1
- If ***p* > α: accept Ho** (because it is likely): reject H_1



Sounds Familiar?

Although there is no significant difference between Control and the three treatments, blood pressure did show a decrease with increasing dose of Drug A

It would be more likely to reject your null hypothesis if:

- The effect is larger
- The sample size is larger
- The α value is larger

Experimental Design:

General Principles
and
Common Pitfalls

Experimental Design: General Principles

1. Control
2. Confounding factors
3. Signal to noise ratio
4. Randomization
5. Error control
6. Treatment and level of treatment
7. Replication & Optimal sample size

Experimental Design: Controls

- Set up proper **Control** to compare with **Treatment** (all conditions in your control should be exactly as those in your treatment(s), except without treatment)
  - Treatment vs No Treatment
  - Before vs After

Question

- How do you set up a proper control if you want to test whether a new Drug can lower blood pressure?

Question

- How do you set up a proper control if you want to test whether a new drug can lower blood pressure?
  - Blind (placebo)
  - Double blind

Experimental Design: Confounding Factors

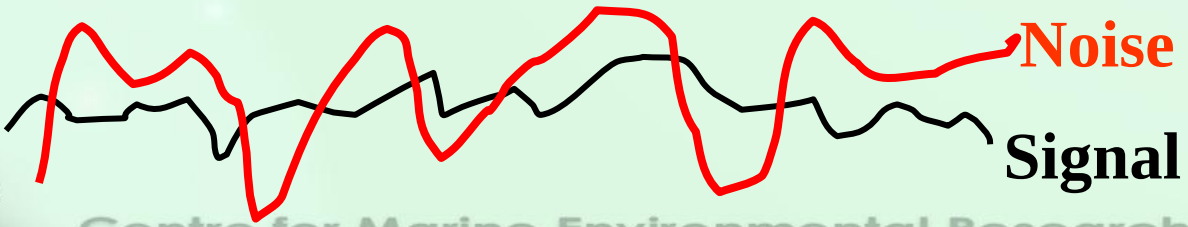
- Confounding factors (e.g. sex, size, different batch/origin of materials) may affect your result
- It may be desirable to control these confounding factors in order to minimize their effects (e.g. use the same sex, same size and same batch of materials in your experiment)

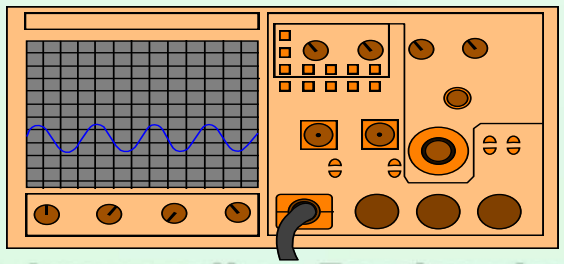
Question: What is the problem in doing this?

Is it a good thing or bad thing?

Signal to noise ratio

- Noise (Natural variations/background)
- Signal (effect that you want to detect)
- You cannot measure any signal if it is less than noise
- If noise is very high:
  - You can only detect very big difference **OR**
  - You have to increase your sample size **OR**
  - You have to reduce noise



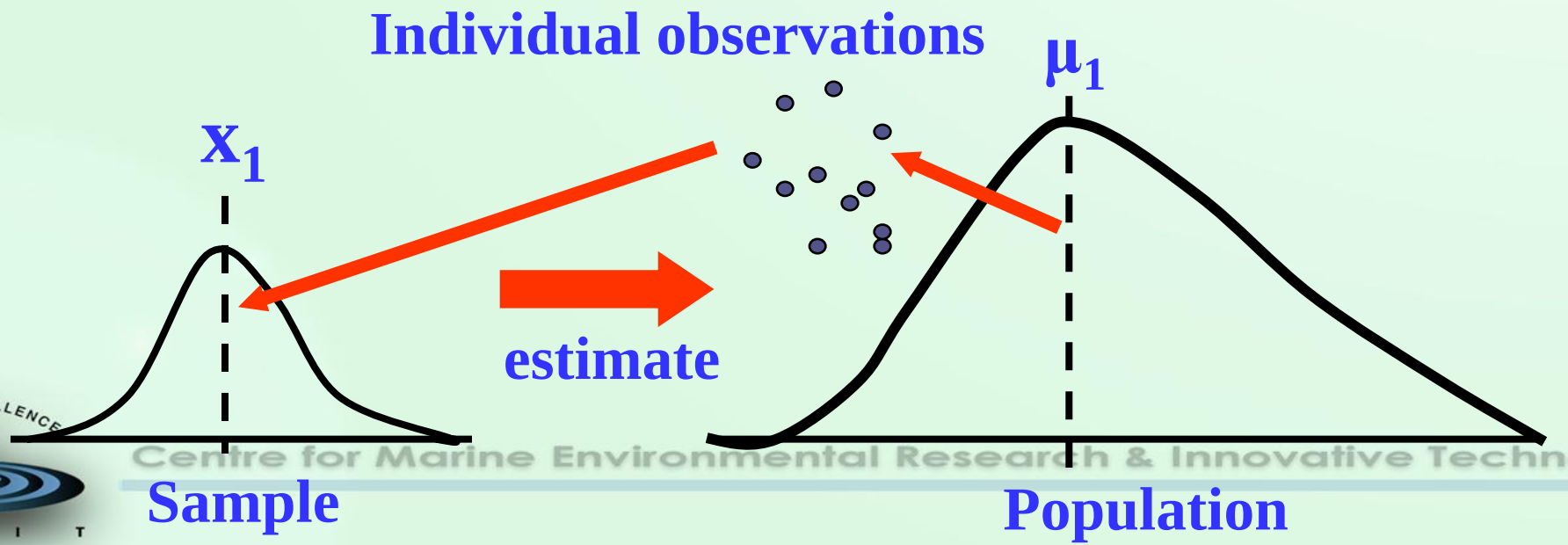


Signal to noise ratio

- If your control= 100±30 mg/L, you can only detect "**signal**" which causes > 30% change
- This is the reason why we always try to control and standardize size, age, source, reproductive stage, tissue (e.g. right lobe of liver) sampling method etc., to minimize **noise** so that we can detect **signal** more easily.
- Noise is generally high in field studies (100-200%), moderate in physiological studies (10%) and low in chemical (2-3%) and physical studies (<0.5%)

Experimental Design: Randomization

- Most (if not all) statistics assumes that samples are comprised of individual observations drawn from the population randomly. Your experimental data may be invalid if this is not so.



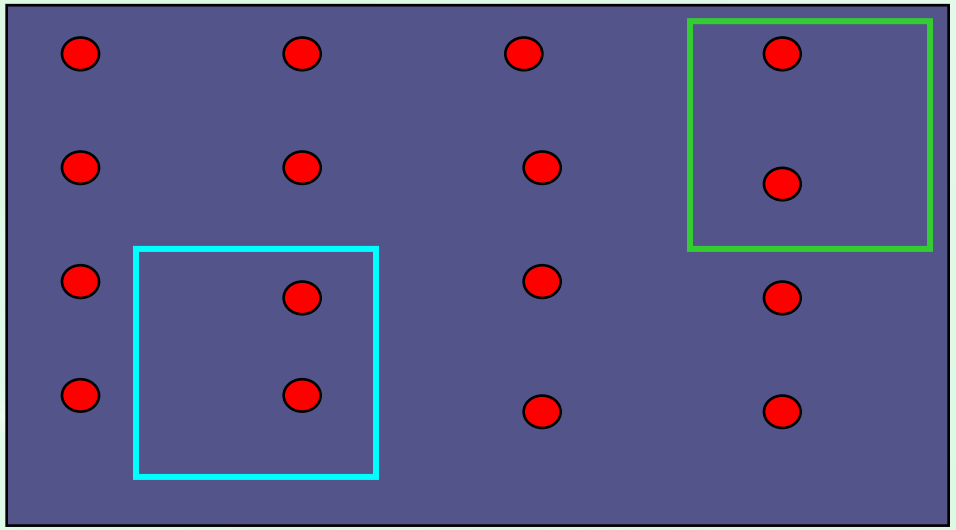
Experimental Design: Randomization

- **Question**: How do you know that you are taking representative samples in your experiment?

- **Question**: How do ensure your sampling is random?

Random Sampling

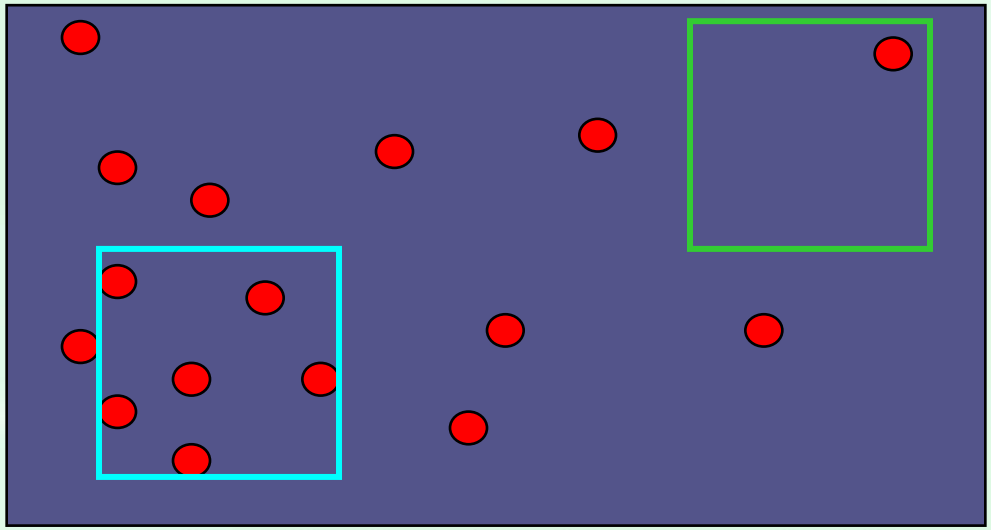
- To ensure that **All** units in the sampling area must have an equal probability of being selected in order to provide an unbiased estimate



Homogenous/uniform Distribution (rare)

Random Sampling

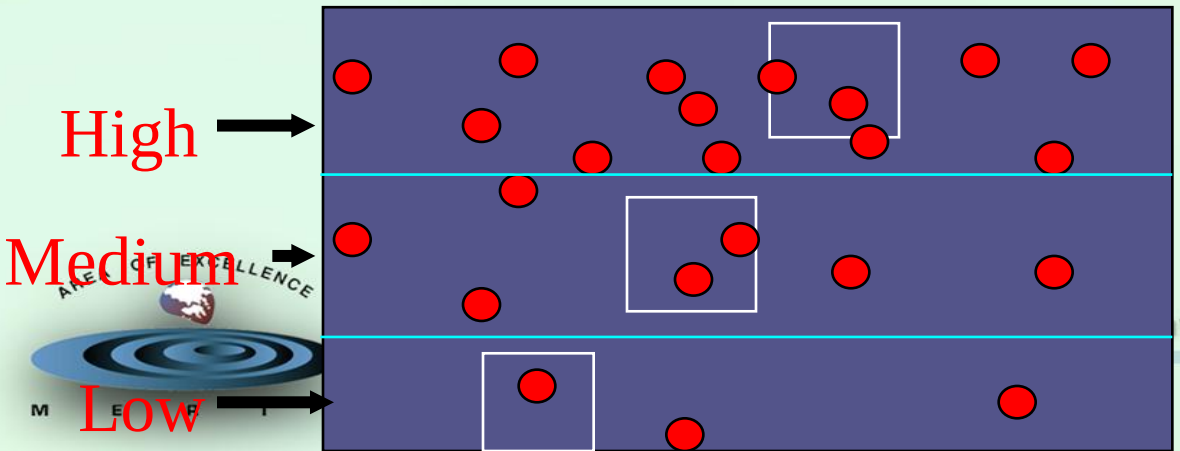
- If the population/distribution is heterogeneous, random sampling is particularly important in order to get an unbiased estimate



Heterogeneous distribution

Stratified Random Sampling

- Divide population into different “strata” and sample each “stratum” independently and randomly
- Sampling fraction in each strata proportional to that of the total population (e.g. sample 3 males and 2 females from a population with of 60% male and 40% female stratum)
- Strata should be included as a predictor variable in the model
- This can produce a weighted mean with less variability than the mean of a random sample of the population and improves the representativeness of the sample by reducing sampling error.

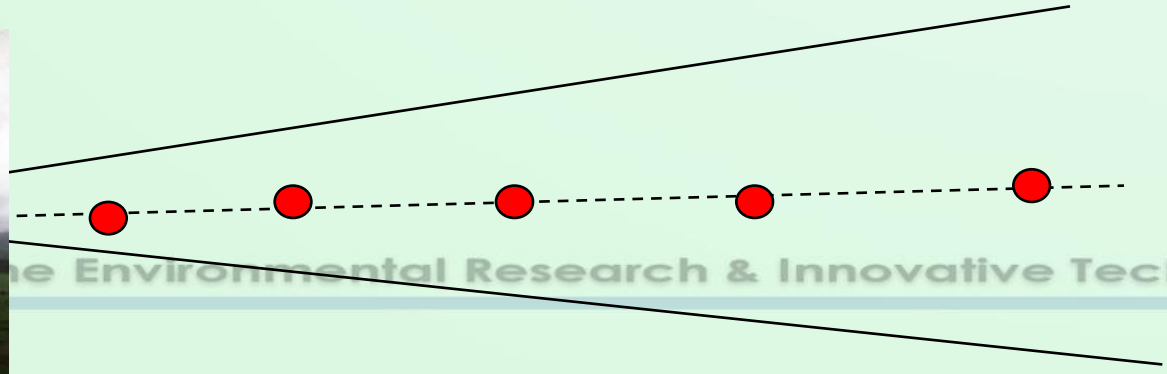


$$\bar{Y} = \sum_{h=1}^{1} W_h Y_h$$

Where: W=proportion of total units in stratum h, Y_h is the mean of stratum h

Systematic Sampling

- Equally spaced
  - Spatial: e.g. plot each 10 km along a transect
  - Time: e.g. every 10 days
- Interested in changes along a gradient
- Run into risk with an unknown gradient



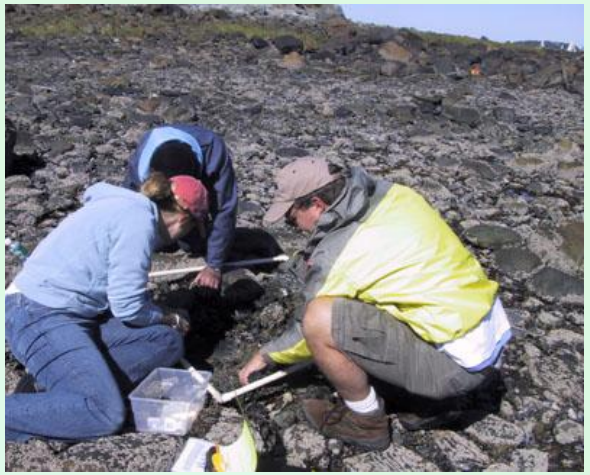
Experimental Design: Error Control

- In any experiment, it is essential to
  - Identify the major sources of variability of your data, and
  - bring the variability under control

Have you done this in your experiment?

Error control

Example 1: Estimate number of intertidal animals using quadrates



Error control

- Different shores 10-1000 (100 times)
- Different tidal level 10-500 (50 times)
- Different quadrates 10-50 (5 times)

There is no point to count animals accurately within each quadrate. You should spend more effort in sampling different shores

Error control

Example 2: Compare mercury levels in fish sampled from a polluted site and a clean site

- Sites 50%
- Individuals 7%
- Tissue 200% (Liver =50X in muscle)
- Sample 1%
  - You cannot detect any difference if you analyze the whole fish (because data will depends on the size of the liver).
  - You can save some effort by pooling the same tissue from different fish for Hg analysis
  - There is little merit to refine your analytical technique or in using high resolution equipment

Error control

You should always concentrate your effort in controlling large error. You may neglect small errors

Consumption= Growth+Respiration+Excretion+feces

100% = 37% + 50% + 3% +10%

There is no need to measure excretion. Instead, spend most of your effort in providing a more accurate estimate on respiration.

Error control

- If you want to compare concentration of mercury in fish from three different sites, error may derive from different:
  - Sites
  - Water depth
  - Species
  - Size
  - Season
  - Individuals
  - Tissues

Error control

If you: (a) only afford to do a fixed no. of samples (say 50), and (b) have some idea about the variations associated with each sampling level, hierarchical sampling design can help you to optimize no. of samples amongst the various levels to minimize error and give you the max. power:

- Water depth 12
- Species 20
- Size 5
- Season 2
- Individuals 8
- Tissues 3

Error Control by Randomization

Experiment: Effects of nutrients on plant growth

1	2	3	4	5
1	2	3	4	5
1	2	3	4	5
1	2	3	4	5
1	2	3	4	5

Put 1-5 times of nutrients in flower pots and measure growth after 1 week

Randomize Block

1	1	1	1	1
2	2	2	2	2
3	3	3	3	3
4	4	4	4	4
5	5	5	5	5

4	1	2	3	1
2	5	5	4	3
5	1	4	2	5
1	4	2	3	1
2	3	5	4	3

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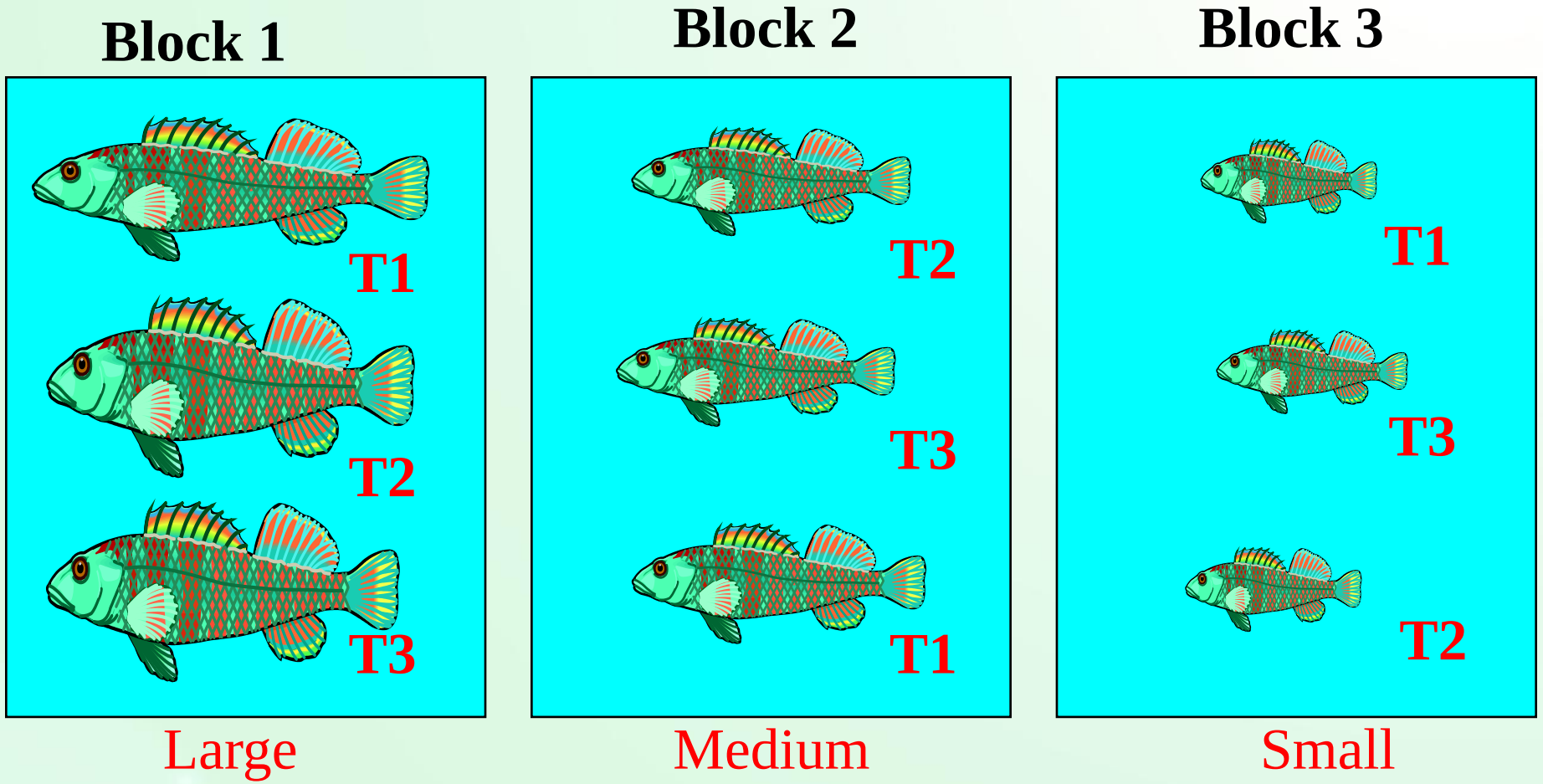
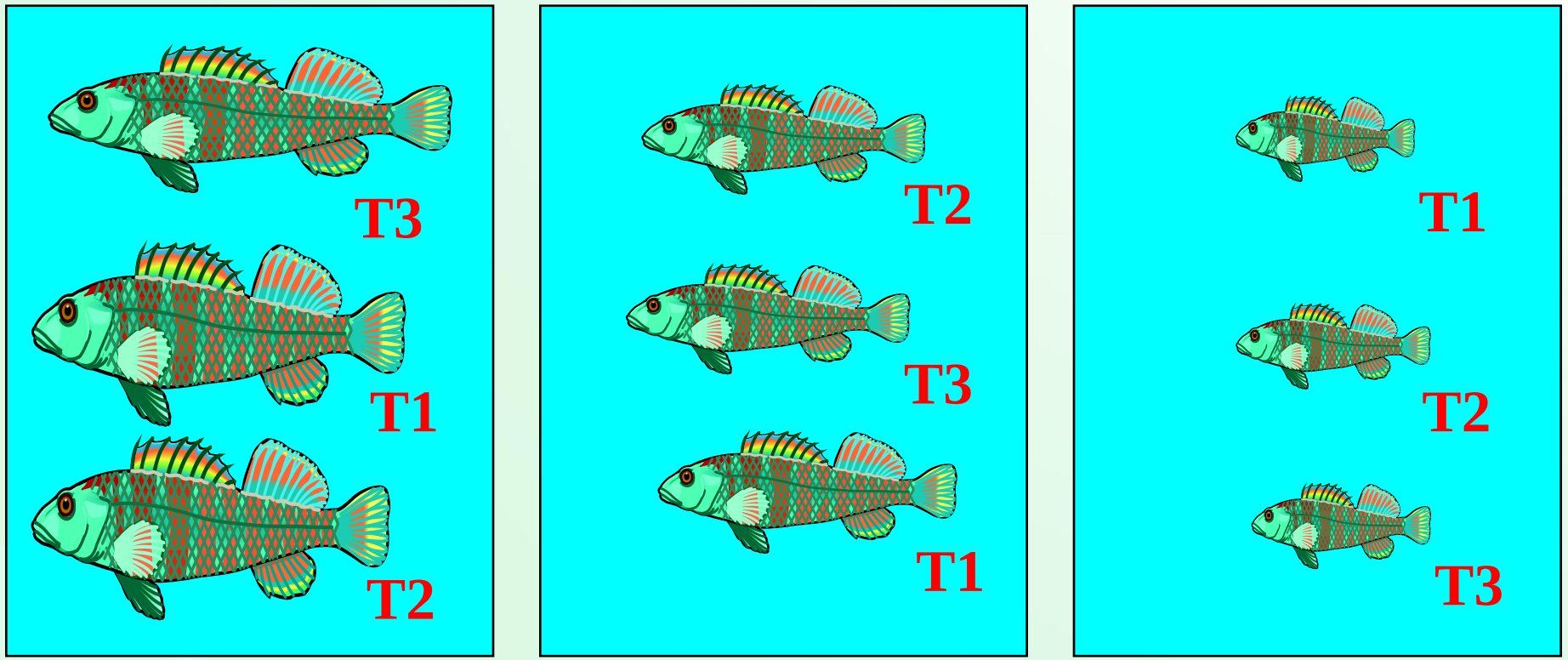
Randomized Block Design



- Group fish in blocks according to their size ("alikes")
- Assign treatment (T1-T3) at random to individuals within each block
- This reduces Within SS more than Within df, (-MS within), thus more likely to reject Ho

Double Randomized Block (Latin Square) for experiments with two obvious sources of variability



Assign treatment (T1-T3) at random to individuals so that no treatment appears more than once in each row or column

Double Randomized Block (Latin Square) for experiments with two obvious sources of variability

			Column		
		1	2	3	4
Row	1	C	D	B	A
	2	B	A	C	D
	3	D	C	A	B
	4	A	B	D	C

Column= 1st Blocking factor ; Row=2nd Blocking factors

Treatment= A, B,C,D

Levels of Treatments and Replicates

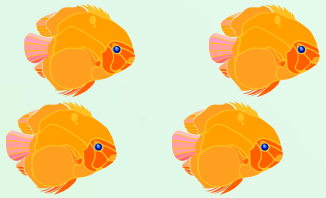
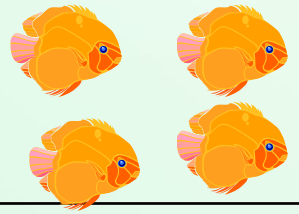
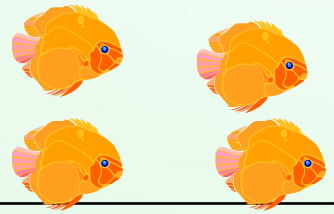
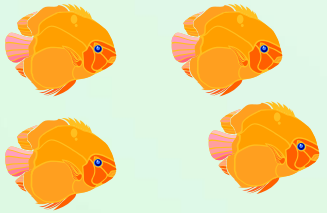
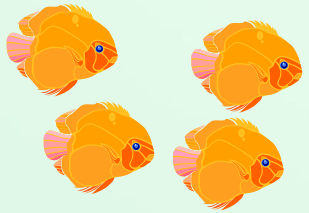
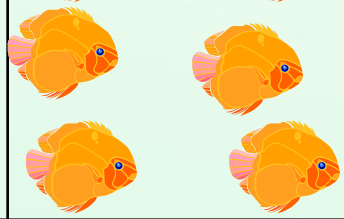
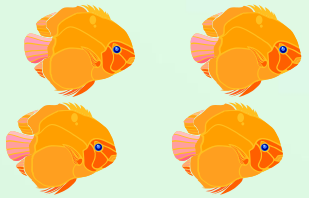
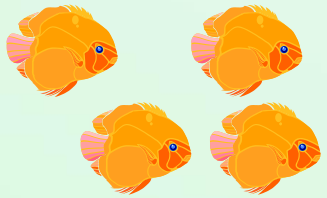
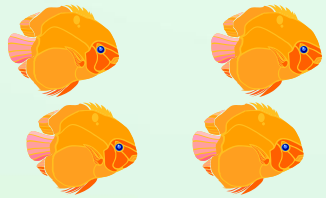
Levels of Treatment & Replication

Treatment	Salinity (o/oo)			Temperature (°C)		
Level	30	20	10	35	25	20
Replicate	5	5	5	5	5	5

Factorial Design

Salinity

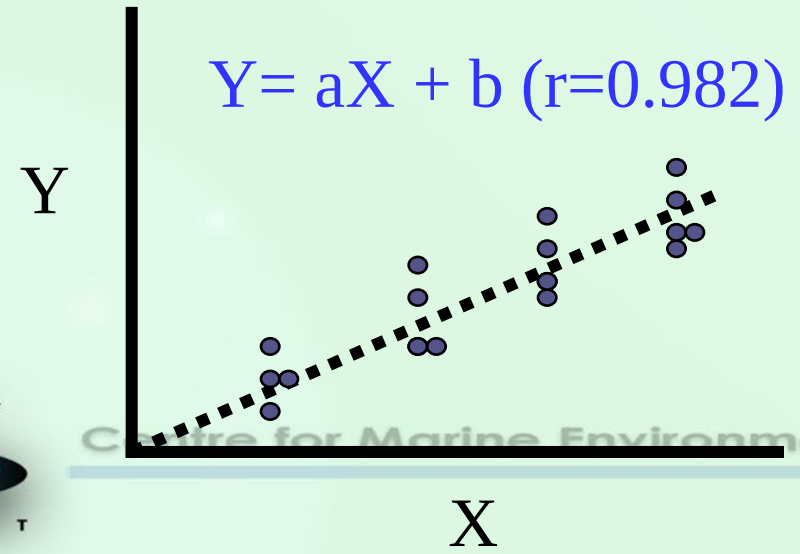
Temperature

	30 o/oo	25 o/oo	20 o/oo
30 °C			
20 °C			
10 °C			

Test three H_o by 2 way ANOVA all in one go:
salinity, temp and interactions (salinity x temp)

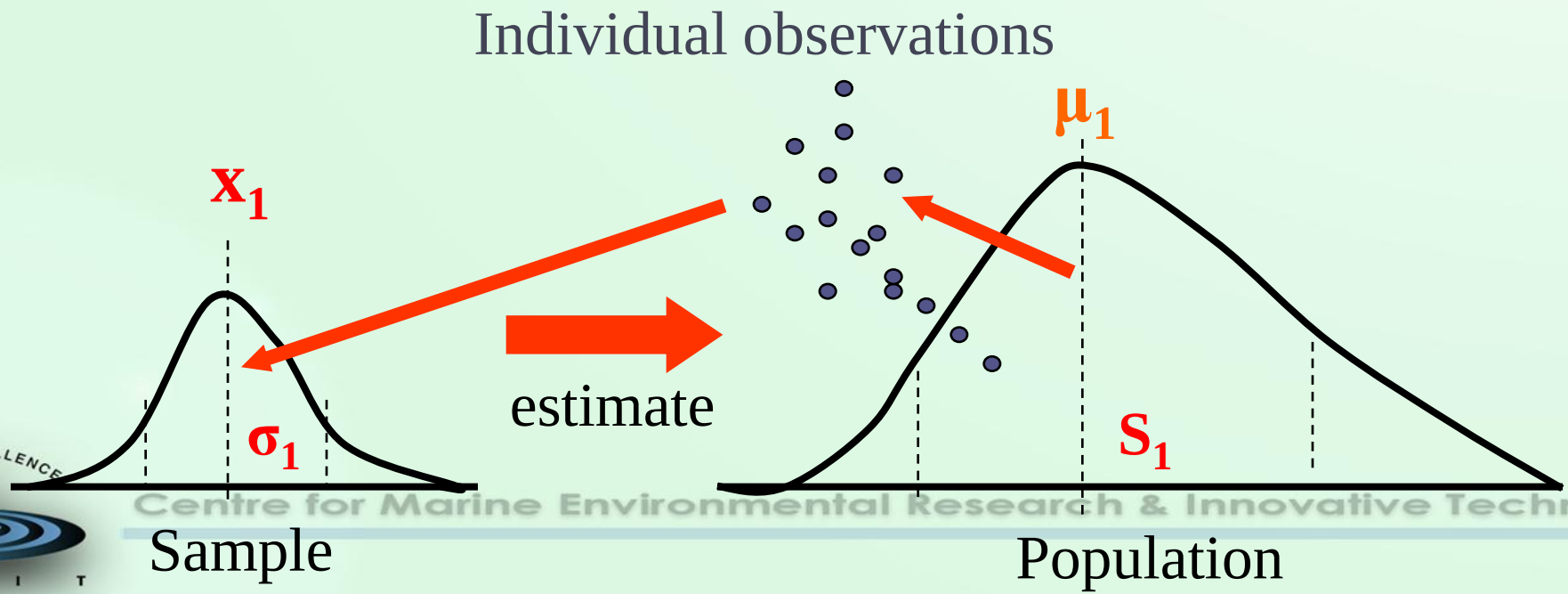
Levels of treatment

- Do you really need different levels of treatments? (Are you really interested in finding out the correlation between X and Y? or predicting Y from X?)
- How many levels of treatments are required? (at least 4-5 for correlation/regression)



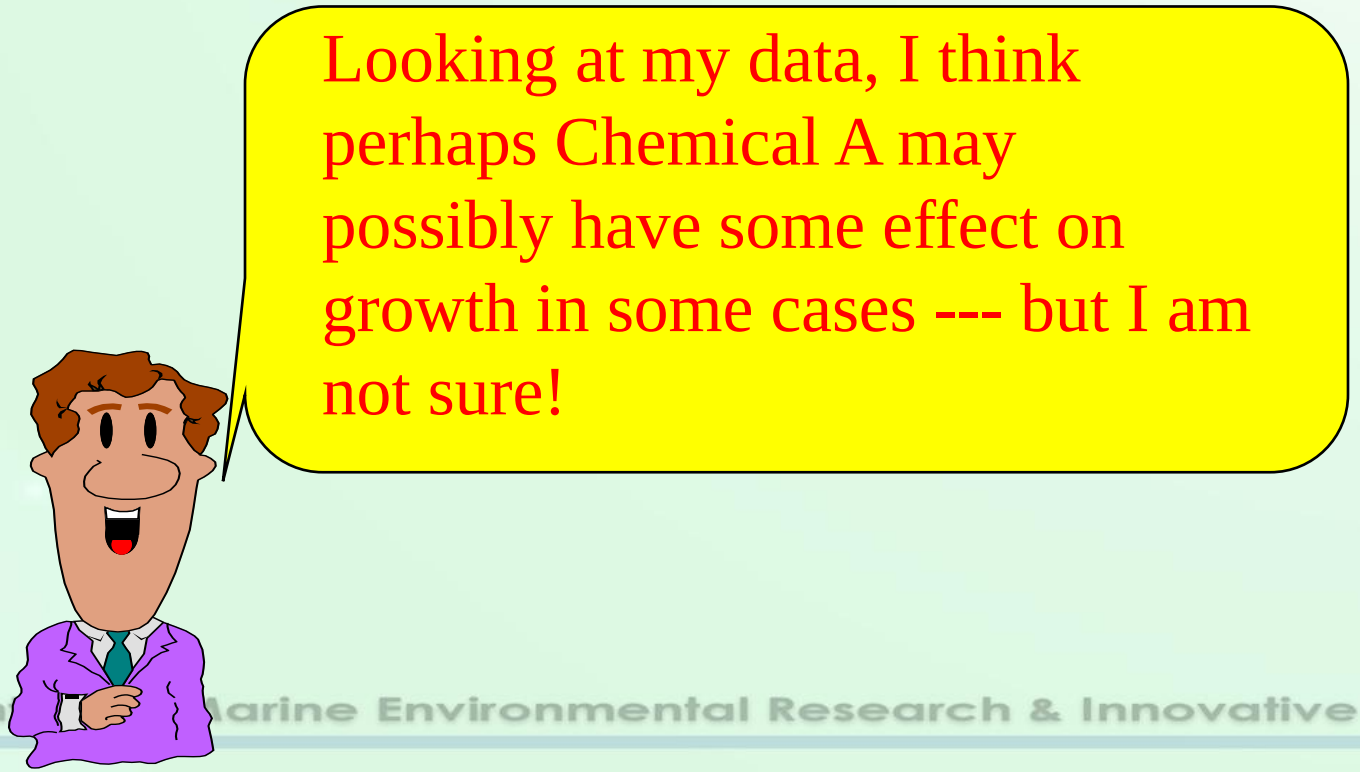
Experimental Design: Replication

- As the no. of individual observations (no. of replicates) increases → X_1 and σ_1 will get closer to μ_1 and S_1



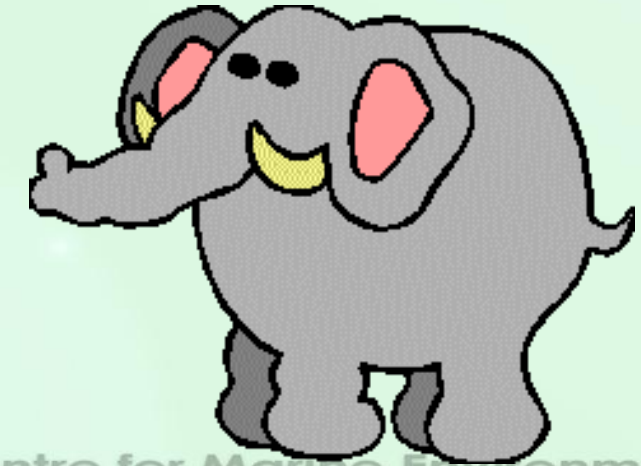
Replication

- **IMPORTANT: NEVER sacrifice no. of replicates**: This may make your experimental results invalid



How many replicates we should have?

- Depends on:
  - Variance among treatments (SS among, signal)
  - Variance within treatment (SS within, noise)
  - How big a difference you want to detect



σ

Coefficient of Variations (CV)

- Variability can be conveniently measured by Coefficient of Variation (**CV**), which is a normalized value of dispersion of a probability distribution, defined as the ratio of standard deviation (**σ**) to the mean (**μ**)

$$CV = \sigma / \mu$$

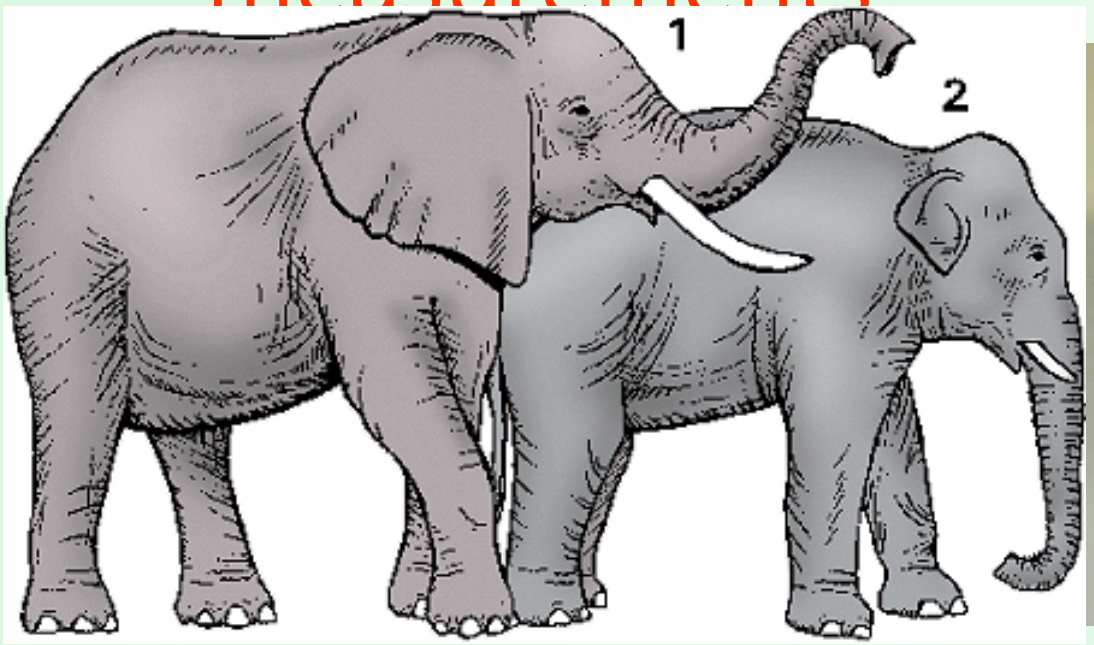
An Example: Within variance of POPs in the marine environment

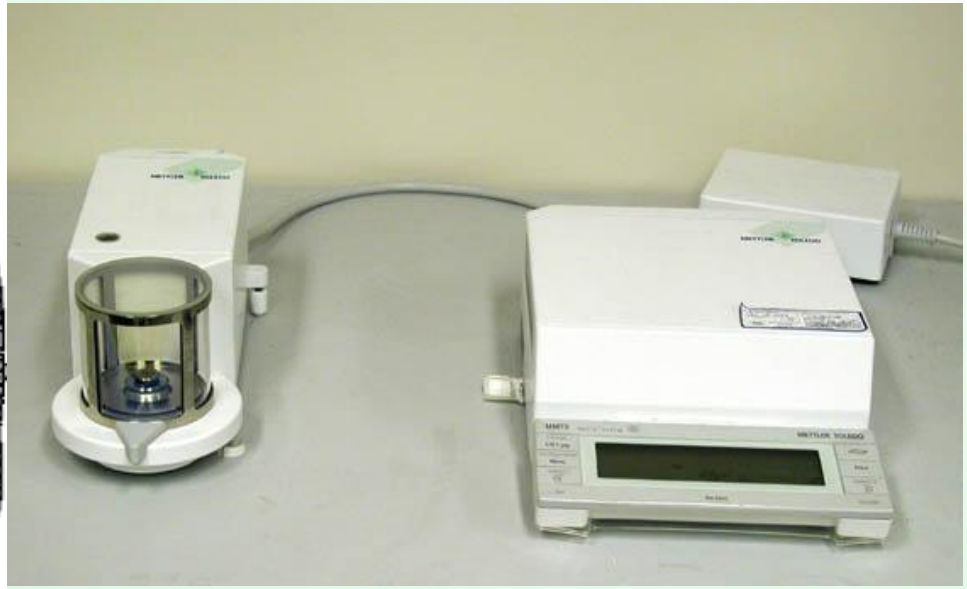
Sample	Chemical	n	Mean ± SD	CV	Reference
Sediment	**PAHs**	**12**	**593 ± 284 ng/g**	0.48	Bouloubassi et al. 2006
Soil	**DDT**	**5**	**0.32 ± 0.47 mg/kg**	1.47	Gaw et al. 2006
Fish	**PCB**	**3**	**110 ± 95 ng/g ww**	0.86	Sethajintanin et al. 2004
Fish	**HCH**	**2**	**0.45 ± 0.21 ng/g**	0.47	Weber & Goerke 2003
Mussel	**PCB**	**4**	**289 ± 253 ng/g**	0.88	Cheung et al. 2002

Source of variations (CV)

	Mean	Median	Maximum	Minimum
Field sampling (n=9)	44.8	39	107	16
Chemical analysis (n=12)	9.9	9	18.8	4.8

Is it necessary to lower the detecting limit or further improve accuracy of measurements?





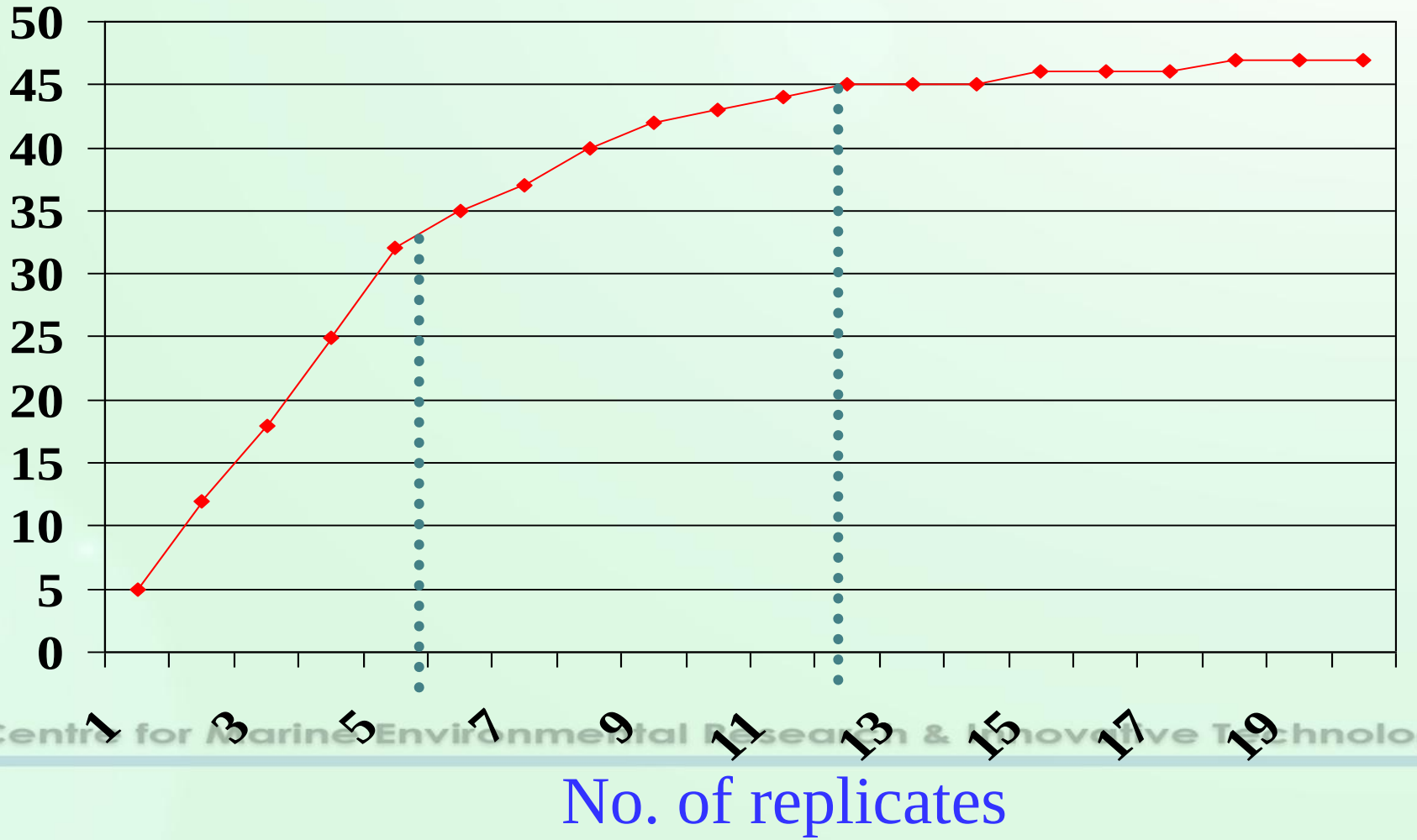
Sediment/soil	Environmental conc.	Current detection limit
DDT	0.005-18.5 ng/g	0.0036 ng/g (EMMI 1997)
PCBs	1.7-124.6 ng/g	0.1-0.6 ng/g (Basheer *et al.*, 2005)
PAHs	0.052-66.7 ug/g	0.01-0.06 ug/g (Gurka *et al.*, 1987)

Based on the above evidence….

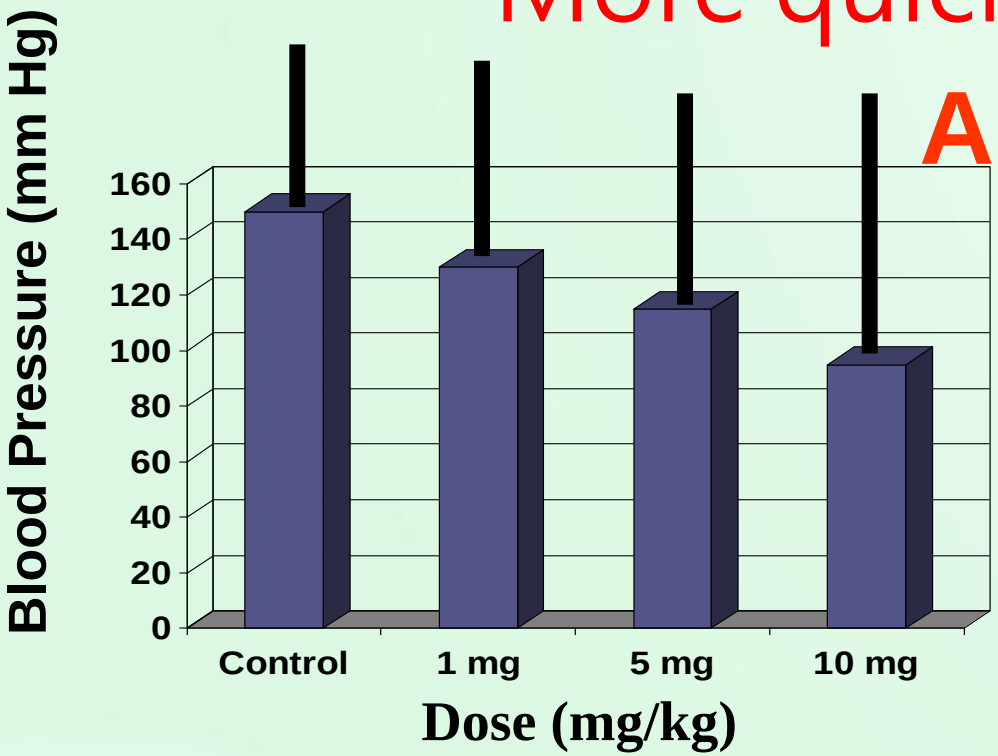
- The major error stems from spatial and temporal heterogeneity, *but not from chemical analysis*. As such, efforts should be devoted to improving sampling design rather than (a) further enhancing accuracy of chemical measurements or (b) lowering detection limit below environmental levels

No. of replicates required : A quick & dirty way

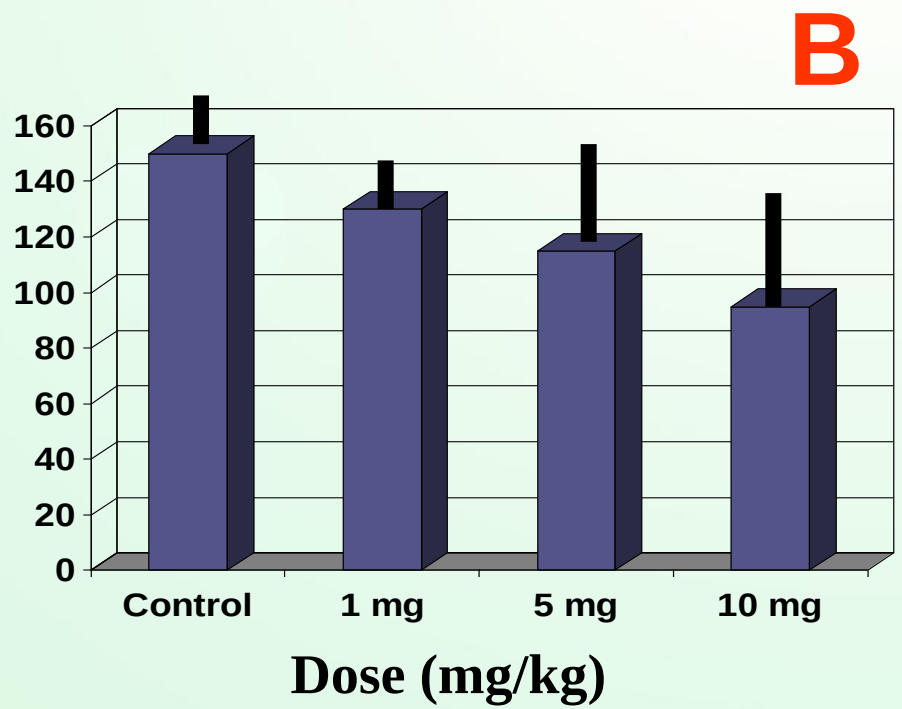
Cumulative No. of species.



No. of replicates required : More quick & dirty way



No significant difference can be found between the treatments and the control



No significant difference can be found between the treatments and the control

Q: What is your insight?

No. of replicates

Length of fish (cm) from different sites

Site A	Site B	Site C	Site D
25	30	32	12
67	12	35	18
18	28	32	16
35	20	35	14
24	22	33	17

Question: Which data set above (A,B or C,D) requires a larger no. of replicates? Why?

No. of replicates

Length of fish (cm) from different sites

Site A	Site B	Site C	Site D
25	30	32	12
67	12	35	18
18	28	32	16
35	20	35	14
24	22	33	17

More replicates are required to detect difference between Sites A & B (than detecting difference between Sites C & D) because variance (within) of A,B is larger than variance (among) of A,B

BUT: Exactly how many replicates are required in each case?

No. of replicates depends on

- Effect size: the magnitude of the effect you want to detect (nothing to do with statistics: this varies between studies, depends on cost-effectiveness, scientific significance and your professional judgment)
- How variable is your data (σ)
- Level of statistical significance (α)
- What statistical test you use

Optimal sample size

$$t = \frac{X_1 - X_2}{\sqrt{\frac{S_1}{n_1} + \frac{S_2}{n_2}}}$$

Where:

X= mean; S=variance; n=no. of replicates

We will reject Ho if t (calculate) > t (tabulate)

Optimal sample size

$$t = \frac{d}{\sqrt{\frac{2Sc}{n}}} \qquad n \geq \frac{2\, t^2_{tab}\, Sc^2}{d^2}$$

Where:

d= difference that you want to look for,
Sc=common variance n=common sample size

Optimal sample size

Suppose:

d=1, Sc= 3.16, n=10

$$\mathbf{10} \geq \frac{2\,(2.1)^2\,(3.16)}{1^2}$$

$10 \geq 28$?? (NOT TRUE)

Optimal sample size

Let's try n=26

$$26 \geq \frac{2\, t^2_{(df=50,\, p=0.05)}\, (3.16)}{1^2}$$

26 ≥ 25.6 (YES!!)

Therefore n < 26 is not enough,

n > 26 is waste of time and effort

Optimal sample size

- For any large scale experiment, it pays to conduct some preliminary experiment to estimate the common variance beforehand
- You have also to decide on:
  - how large a difference that you want to look for
  - how many samples that you can afford to do

Rule of thumb for no. of replicate

- If you need a very large no. of replicates, you may well be looking for a difference that has trivial scientific significance
- df < 5: only large difference can be detected
- df>30: further increase in sample size probably won't help
- Fewer replicates are required in factorial design experiments

Question:
Is α =0.001 better than α =0.05?

Type I error & Type II error

		Truth	
		H_O	H_1
Decision	H_O	Accept Ho when it is true (Good)	Type II Error (β : False positive)
	H_1	Type I error (**α**:False negative)	Reject Ho when it is false (Good)

Power= (1- ß): The probability of correctly rejecting Ho when it is false (i.e. detecting a real effect)

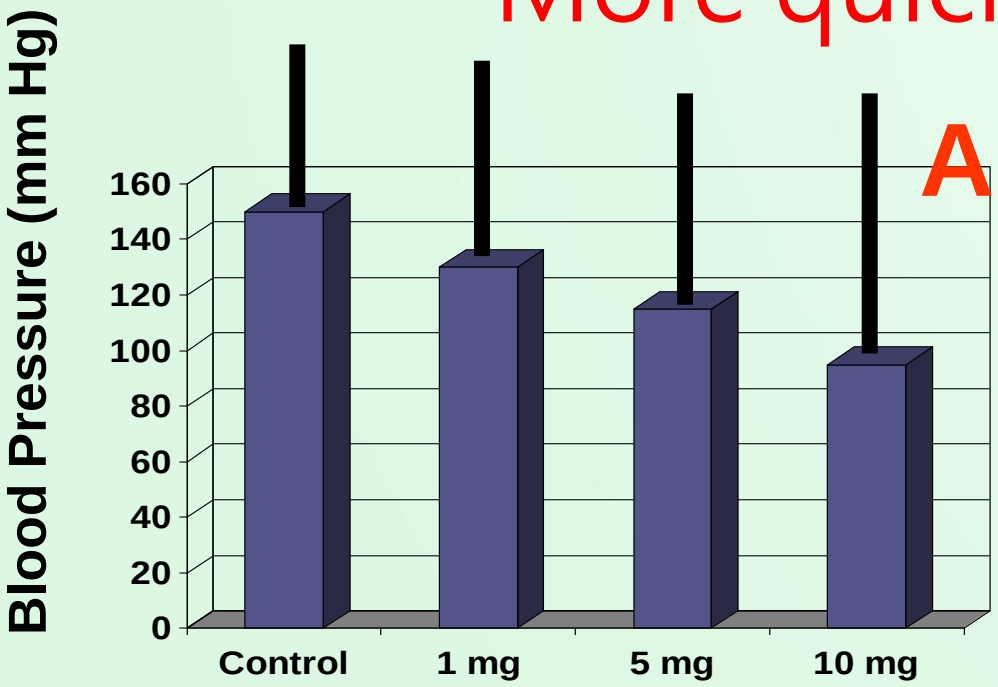
Power Analysis

- By reducing α (say, from .10 to .01), we reduce the likelihood of a Type I error BUT increase the likelihood of a Type II error.

Power analysis

- If test result is not statistically significant, there are two possibilities:
  - there is no real effect (That's good!)
  - your study design could not detect the real effect (That's bad!!)
- Power analysis helps you to distinguish between these alternatives

No. of replicates required : More quick & dirty way



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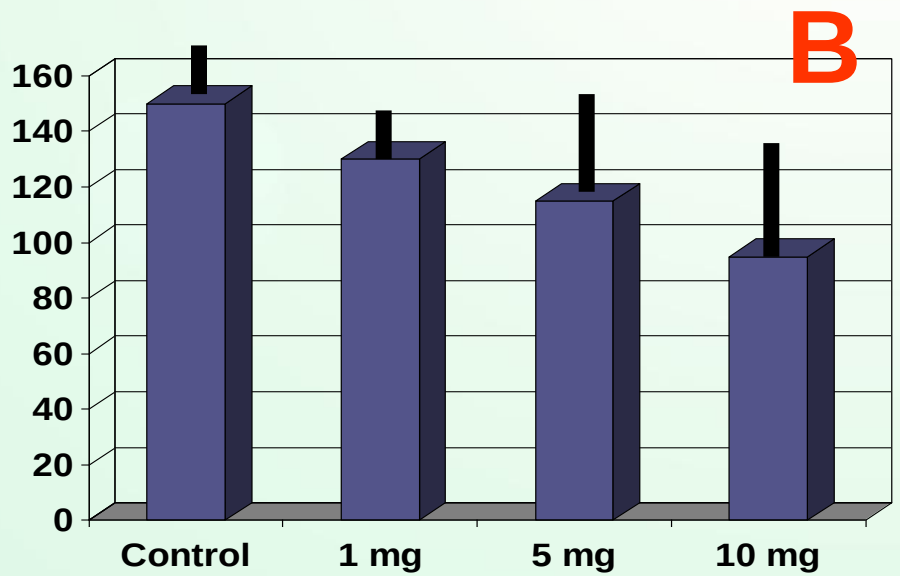


No significant difference can be found between the treatments and the control

Q: What is your insight?

Power analysis

- Enables you to:
  - determine the probability of getting a statistically significant result given that the effect is real
  - work out how small a change that you can detect
  - No. of replicates required (given power, variance, significant level, effect size known)

Power is related to

- How big is the change (ES: Effect Size)
- Sample size (n)
- Variance (σ^2)
- Significant level (α)

$$\text{Power } \alpha \frac{\text{ES } \alpha \sqrt{n}}{\sigma}$$

G*Power: Free!!!

http://www.power-analysis.com/index_.html?gclid=CJWwzsDetawCFaWL4goduyDLHA

Bad experimental design: Some real examples

What is the chance of detecting a 20% difference if *n* = 2, 5 & 10?

Samples	Countries/ Regions	∑POPs	Average CV	Probability of detecting a 20% difference		
				n = 2	*n* = 5	*n* = 10
Sediment	France	PAHs	0.44	6%	12%	22%
	New Zealand	DDTs	1.07	3%	4%	5%
Water	Spain	HCBs	0.39	7%	15%	28%
Fish	Salton Sea, US	PCBs	0.23	17%	40%	71%
	Salton Sea, US	HCHs	0.25	14%	34%	63%
	Oregon, US	PCBs	0.56	5%	8%	14%
	Antarctica	HCHs	0.16	33%	72%	96%
	Antarctica	DDEs	0.24	15%	37%	67%
Mussel	Hong Kong	PCBs	0.69	4%	6%	10%

M E R I T

>50% chance

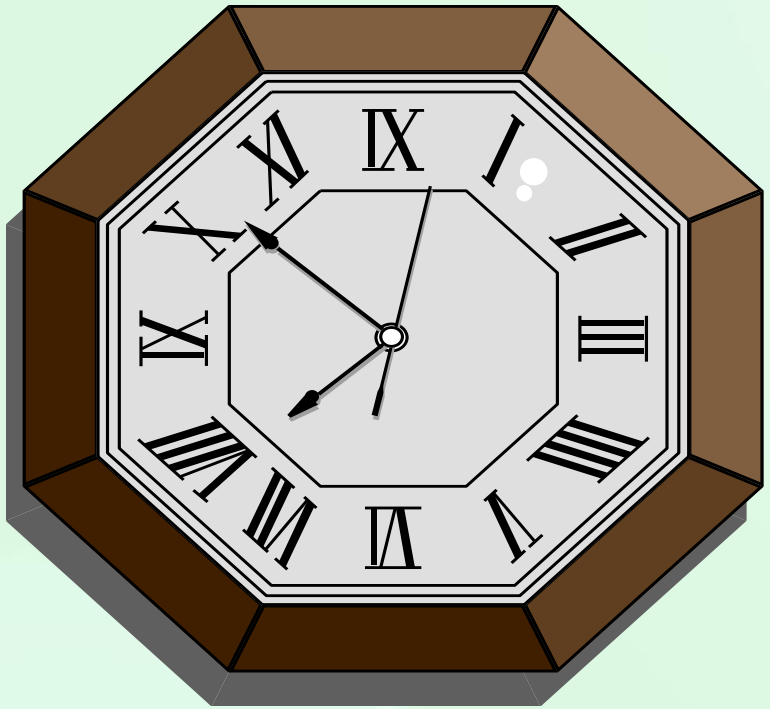
How large a difference we can detect with 80% of chance if *n* = 2, 5 and 10?

Samples	Countries/ Regions	∑POPs	Average CV	% difference (δ) detectable with 80% probability		
				n = 2	n = 5	n = 10
Sediment	France	PAHs	0.44	448%	74%	44%
	New Zealand	DDTs	1.07	1089%	181%	108%
Water	Spain	HCBs	0.39	397%	66%	39%
Fish	Salton Sea, US	PCBs	0.23	234%	39%	23%
	Salton Sea, US	HCHs	0.25	255%	42%	25%
	Oregon, US	PCBs	0.56	570%	95%	57%
	Antarctica	HCHs	0.16	163%	27%	16%
	Antarctica	DDEs	0.24	244%	41%	24%
Mussel	Hong Kong	PCBs	0.69	703%	117%	70%

< 30% difference

Choosing your α and β

- Generally accept α=.05 and β=0.2 (80% power)
- This implies that type I error is 4 times as "harmful" as type II error (α : β= .05 : 0 .2): But no basis at all !!!
- You should strike a balance between α and β to suit your need, e.g.:
  - In screening a new drug, you may set α=.20 and power at 95%, to ensure that a potentially useful drug is not overlooked.
  - In studying side effects of a drug, you may set α=.01 while keeping power at 95%, to better detect harmful effect



Thank you

An Example: Within variance of POPs in the marine environment

Sample	Chemical	n	Mean ± SD	CV	Reference
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Soil	**DDT**	**5**	**0.32 ± 0.47 mg/kg**	1.47	Gaw et al. 2006
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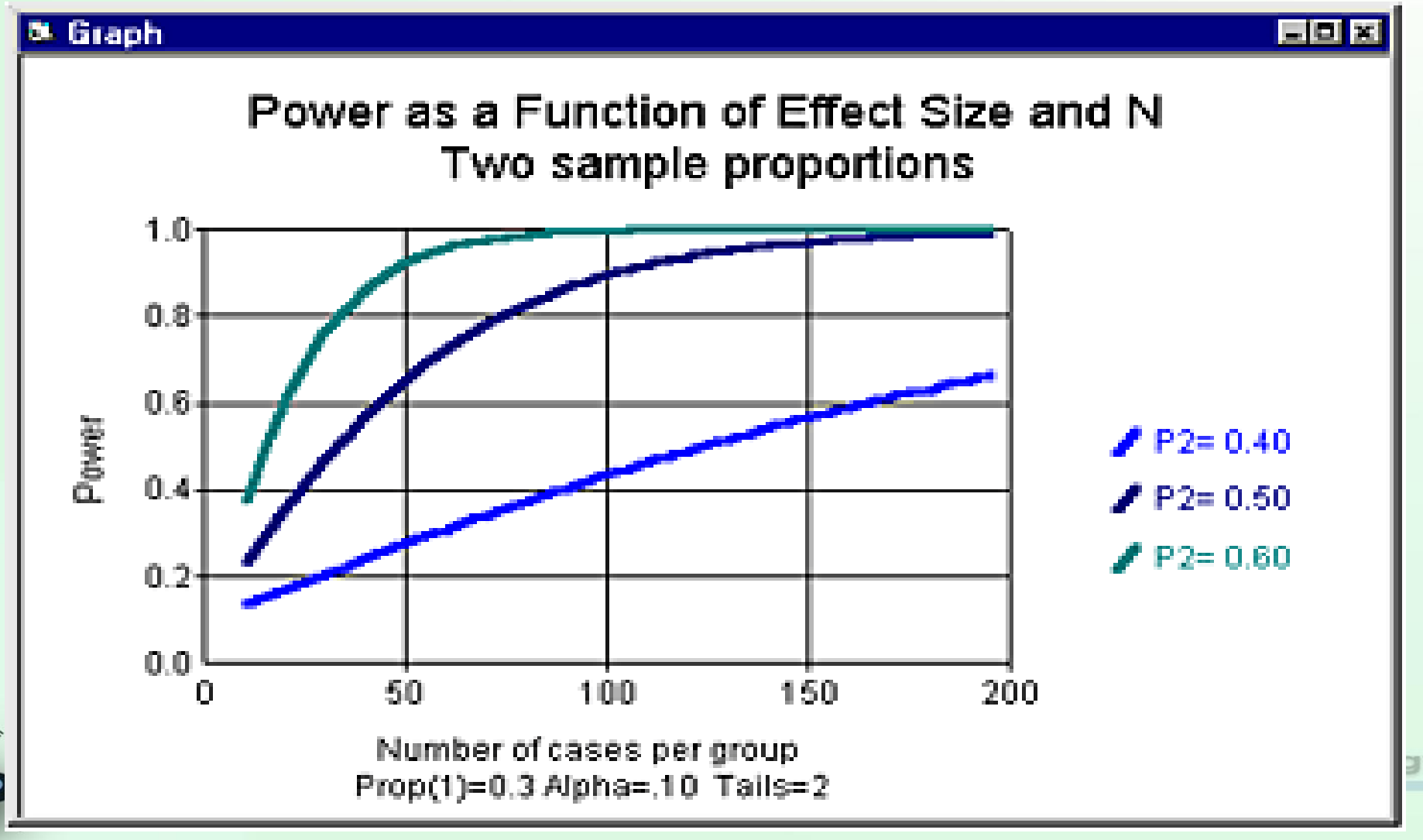
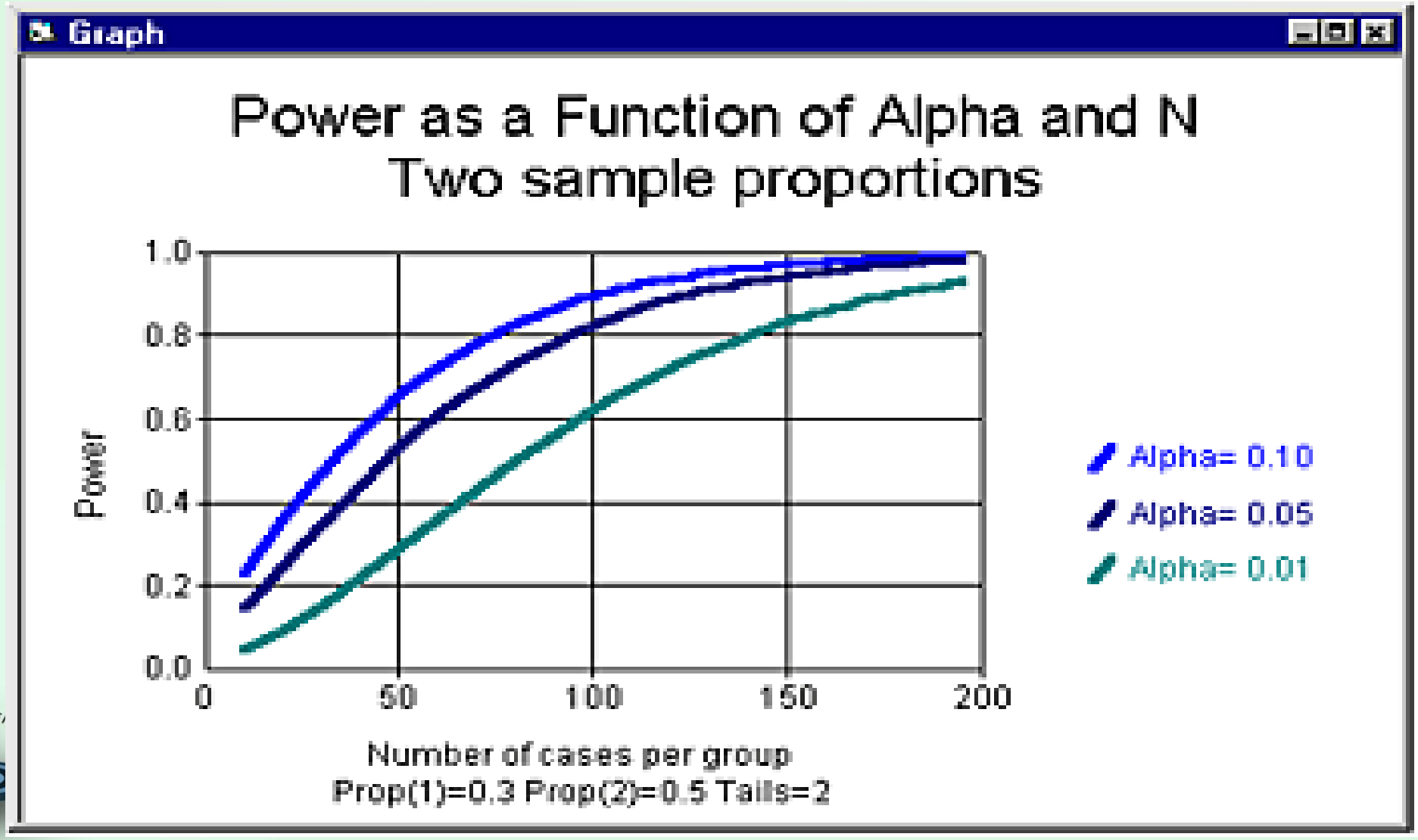
Mussel	**PCB**	**4**	**289 ± 253 ng/g**	0.88	Cheung et al. 2002


Graph
Power as a Function of Effect Size and N
Two sample proportions
Power
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
Number of cases per group
Prop(1)=0.3 Alpha=.10 Tails=2
P2= 0.40
P2= 0.50
P2= 0.60


Graph
Power as a Function of Alpha and N
Two sample proportions
1.0
0.8
0.6
0.4
0.2
0.0
Power
0
50
100
150
200
Number of cases per group
Prop(1)=0.3 Prop(2)=0.5 Tails=2
Alpha= 0.10
Alpha= 0.05
Alpha= 0.01