Experimental design in environmental assessment

© Environmental sampling and analysis (Quinn & Keough, 2003)

Principles of experimental design

- Minimising confounding
  - replication
  - controls
  - randomisation
- Reducing unexplained variation
- Power analysis (Type II error)
  - determining required sample size
  - interpreting non-significant results

Confounding

- Effect of different factors (predictor variables) cannot be distinguished
- Experiments:
  – treatment effects confounded with (inseparable from) other spatial or temporal differences
  – usually due to inappropriate replication or non-randomisation of treatments to experimental units

Replication

- Biological systems inherently variable
  - particularly ecological systems
- Replication:
  - allows estimation of variation in population
- ANOVA:
  - variation between groups compared to variation within groups (residual)
  - replication allows estimation of residual variation

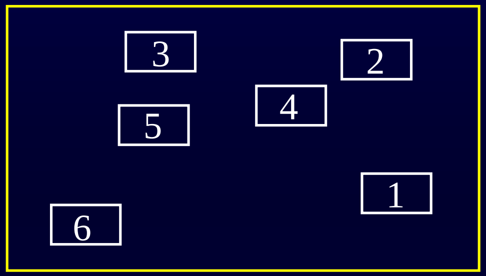
Replication and confounding

- Experimental units (“replicates”) must be replicated at spatial and temporal scales appropriate for the treatments
- Inappropriate replication can result in confounding
  - replicates not at scale of treatments
  - unreplicated at correct scale

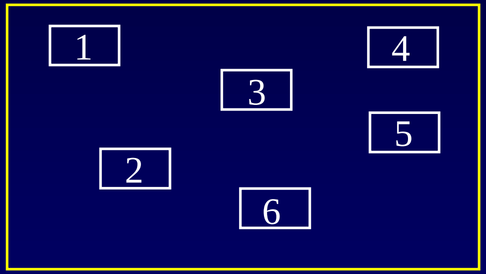
Field comparisons

- One burnt location and one unburnt location surveyed for mammals after fire
- Each location divided into 6 smaller plots
  - mammals sampled from each plot
- Mean no. mammals between locations compared with *t* test

Burnt



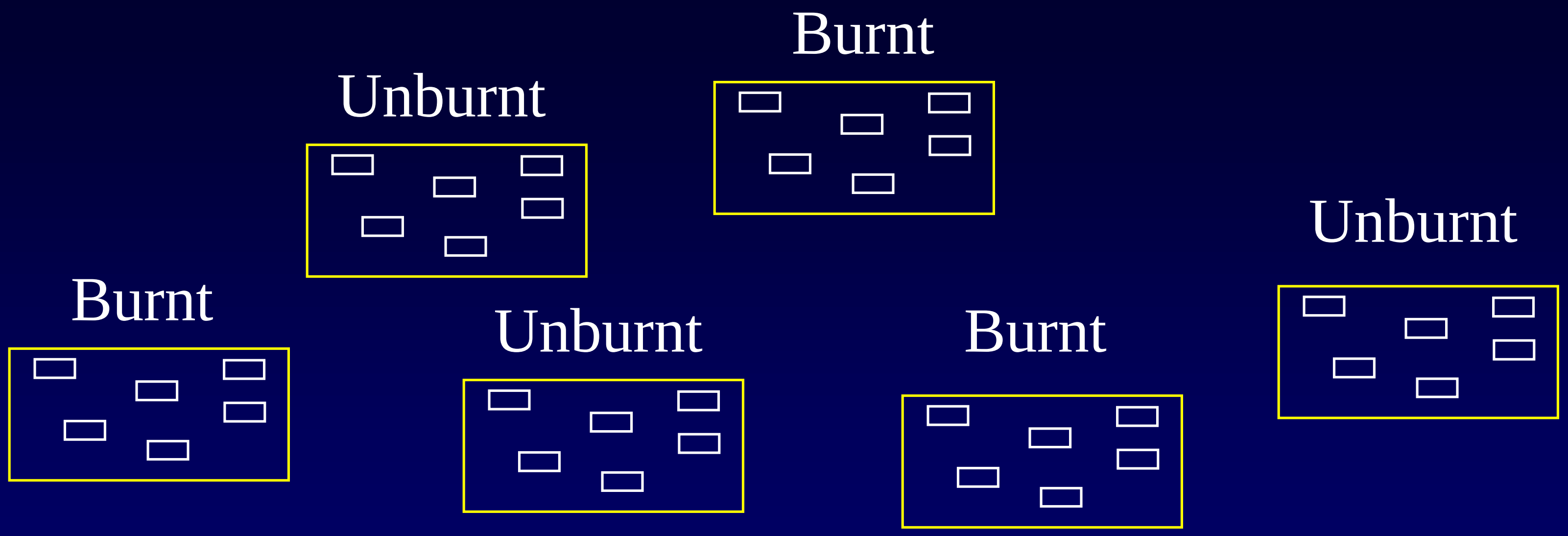
Unburnt



- Burning treatment applied to location, not to small plots
- Plots are only subsamples
  - termed “pseudoreplicates” (Hurlbert 1984)
- True replicates are locations
  - only 1 replicate per treatment

- Locations may be different in small mammal numbers irrespective of fire
- Effect of fire cannot be distinguished from other spatial differences between locations
  - confounding of two factors (burning and location)
  - no conclusions possible about effect of fire

- Experiment requires replicate burnt and unburnt areas



Laboratory experiments

- The effects of uranium wastewater on growth rate of freshwater snails
- Two aquaria set up:
  – one aquarium receives wastewater
  – second aquarium receives equivalent amount of freshwater
  – 20 “replicate” snail in each aquarium
  – size of each snail measured at the start and end of experiment

- Wastewater treatment applied to whole aquaria, not to individual snails
- Snails are only pseudoreplicates
- True replicates are aquaria
  - only 1 replicate per wastewater treatment
- Confounding of wastewater effect and other differences between aquaria

Crossover design

- Run experiment with aquarium one with wastewater and aquarium two without wastewater
- Swap aquarium and wastewater treatment so aquarium one without wastewater and aquarium two with wastewater
  - confounding of wastewater and "aquarium" less likely
- Better but still "unreplicated"

Assessing human disturbances

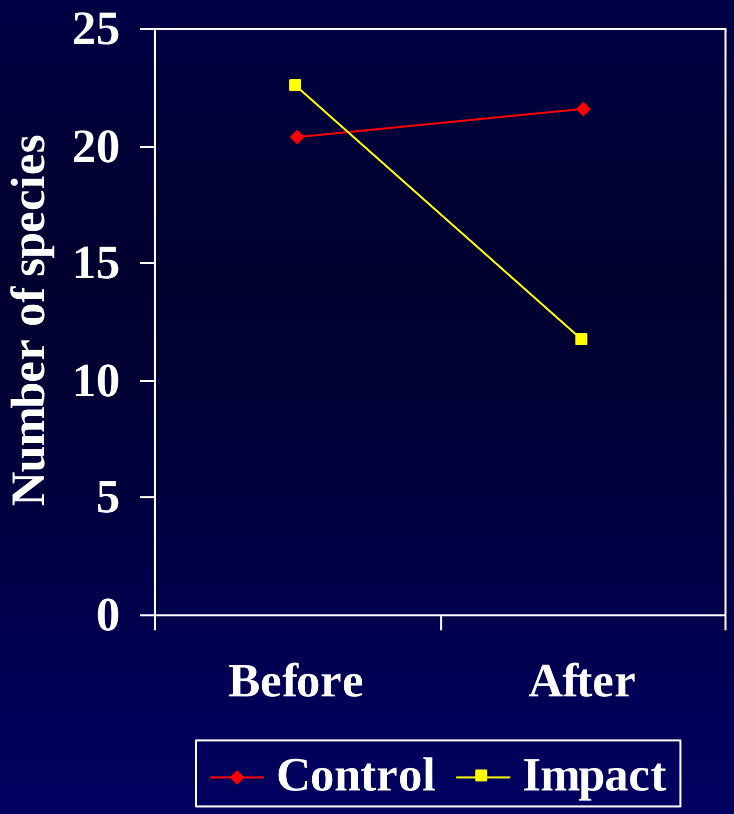
- Sewage outfall on sandy beach
  - "Treatment" beach with outfall
  - Control beach without outfall
- Replicate sediment cores from each beach
- True replicates are beaches
  - only 1 replicate per human impact treatment
- Sewage effect confounded with beach

BACI designs

- Before-After-Control-Impact (BACI) design for impact assessment
- Control and impact locations recorded both before and after impact
- Does control-impact difference change from before to after impact?

BACI designs

- Test:
  – C-I difference changes from before to after impact



Controls

- Many factors that could influence the outcome of experiment are not under our control
  – allowed to vary naturally

- What would happen if the experimental manipulation had not been performed?
  – controls

Salamander competition

- Hairston (1989)
- Hypothesis
  - 2 species of salamanders (*Plethodon jordani* and *P. glutinosus*) in the Great Smoky Mountains compete

Source:
University of Michigan, Museum of Zoology

- Experiment:
  Treatment = *P. glutinosus* removed from plots
  Control = *P. glutinosus* not removed

- Treatment:
  - population of *P. jordani* increased following *P. glutinosus* removal
- Control:
  - population of *P. jordani* on control plots (with *P. glutinosus* not removed) showed similar increase

Laboratory experiments

- Effects of toxicant on survivorship of fish
  - compare response of fish injected with toxicant to response of control animals not injected
- Differences between control and injected animals may be due to injection procedure
  - handling effects, injury from needle etc.

- Suitable control is to inject animals with inert substance
  - handling effects, injury etc. same for control and treatment animals
- Any difference between treatment and control animals more likely due to effect of drug

Field experiments

- The effect of predatory fish on marine benthic communities (eg. mudflats)
  - compare areas of mud with fish exclusion cages to areas of mud with no cages
- Differences between 2 types of areas may be due to cage effects
  - shading, reduced water movement, presence of hard structure etc.

- Must use cage controls
  - cages with small gaps to allow in fish
  - shading, water movement etc. same for cages and cage controls
- Any difference between exclusion and control areas more likely due to effect of fish

Randomisation

- Experimental units must be randomly allocated to treatment groups
- Ensures confounding is less likely

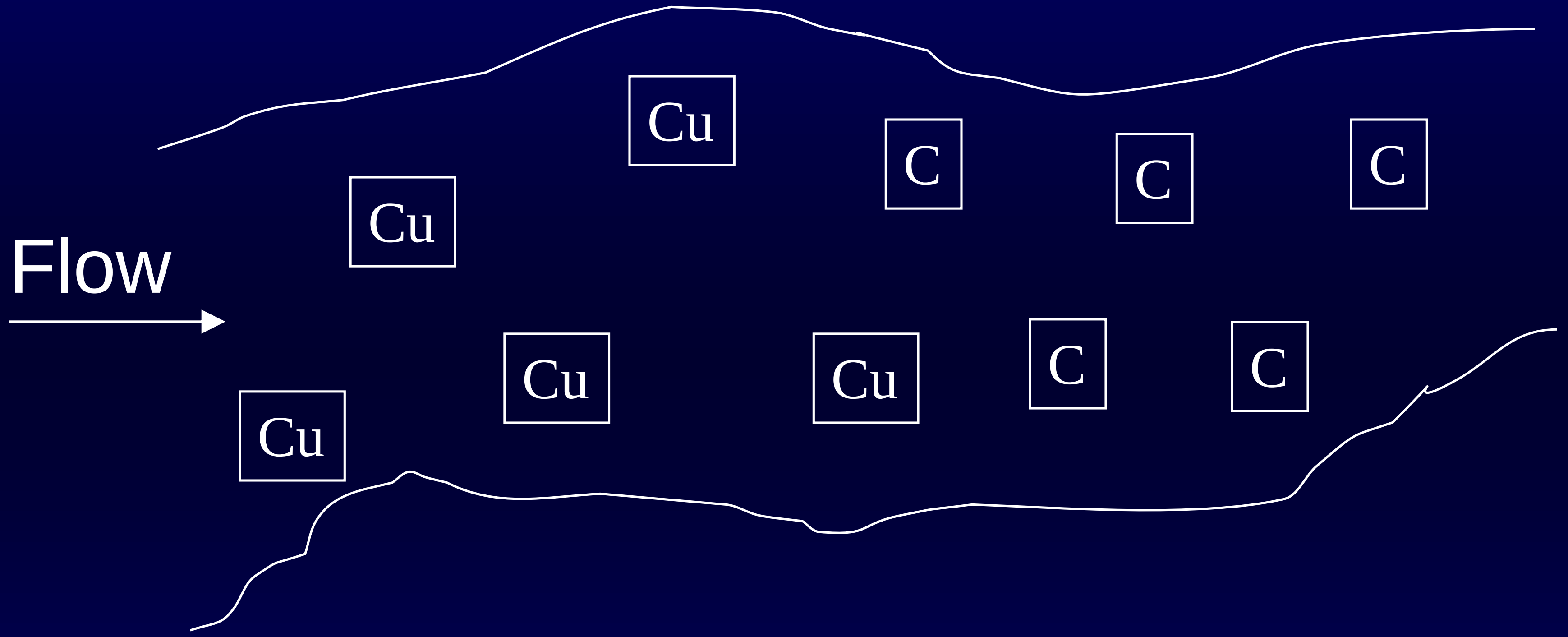
Allocation of replicates to treatments

- Effect of toxicant on [Hb] in blood of flathead
- Fish sampled from population of fish in aquarium
- First six fish caught:
  - used for treatment group
  - injected with toxicant before [Hb] is measured
- Next six fish:
  - used as the controls
  - control injection before [Hb] is measured

- BUT first 6 fish caught are probably slower or more stupid or less healthy, and hence easier to catch
- Effects of toxicant are confounded with health of fish?

Randomisation vs interspersion

- Experiment on effects of copper on stream invertebrates.
  - randomly choose 10 stones in stream
  - randomly allocate 5 as copper treatments (Cu) plots and 5 as control (C) plots
- Possible random arrangement:

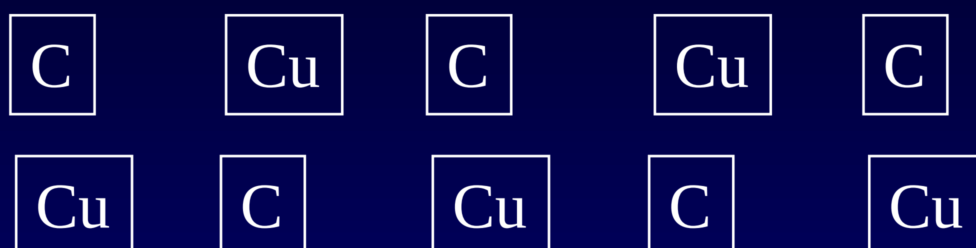


Cu
C
C
C
Cu
Flow
Cu
Cu
C
C
Cu

- Interspersed of treatments important
  - avoids confounding copper effects with other spatial differences
- Re-randomise
  - to get reasonable interspersion
  - decide *a priori* unacceptable degree of spatial clumping
  - single re-randomisation usually improves interspersion

Why not systematic?

Why not arrange the treatments in a systematic way to guarantee perfect interspersion:



Problems with systematic

- Positions of plots not random:
  - not random sample of any population?
- Non-random spacing interval between neighbouring treatments:
  - may coincide with unknown environmental fluctuation
  - confound treatment effects