Essential considerations in designing experiments

1. Carefully considered questions or hypotheses
2. Characteristics / requirements of the "target"
3. Suitable replicates to "overcome the variance"
4. Statistical analyses, planned before the study
5. Suitable controls

May sound straightforward - but can be challenging, even for "simple" questions;

There is no such thing as "the perfect experiment" in biology… only a continual quest toward that goal…

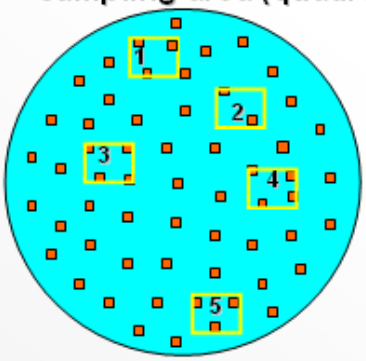
Don Quijote

Essential considerations in designing experiments -

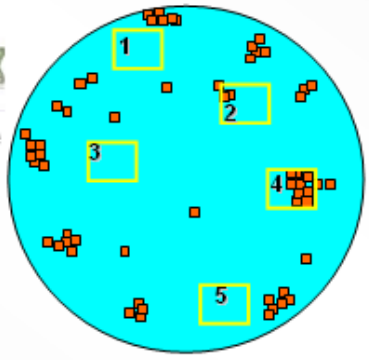
must know the general characteristics of the "target"

The variability among replicates will depend upon the target and the specific environment; easier to assess a uniformly distributed factor, and to be confident about the data.

The truth: 60 fish in this small pond, or 3.3 fish per rectangular sampling area (quadrat).



But fish are usually in groups



Literature/ experts + Statistician

3 replicates → mean 3.00
4 replicates → mean 3.25
5 replicates → mean 3.20

3 replicates → mean 0.67
4 replicates → mean 3.00
5 replicates → mean 2.40

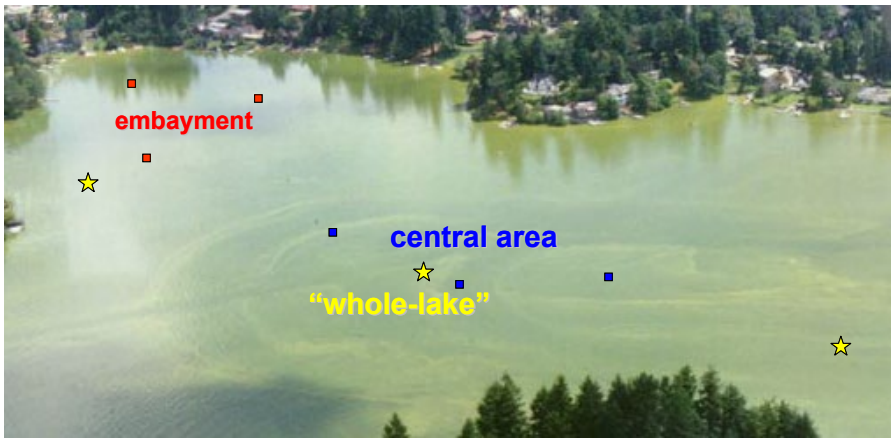
Examples -

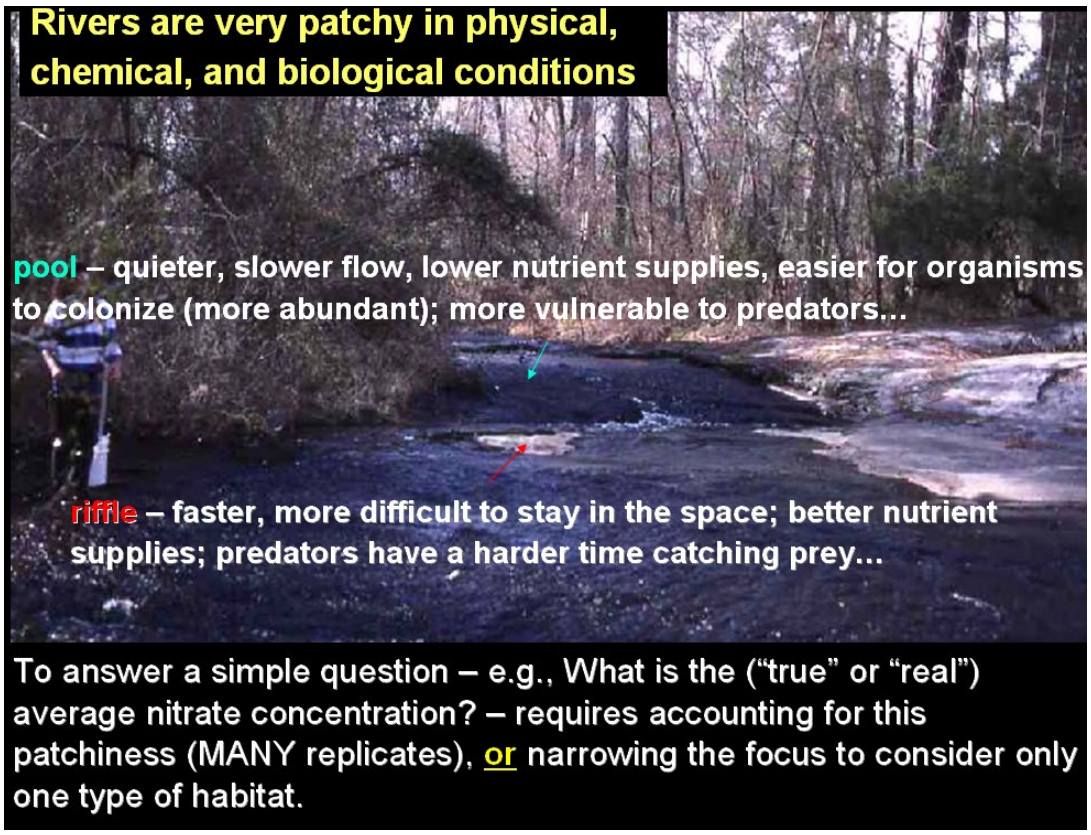
Open-water areas in lakes and reservoirs can appear to be relatively uniform in physical, chemical, and biological features…

But shorelines, and biology, can make lakes patchy

Commonly, scientists do not have the funding or the resources that would make it possible to adequately sample an entire lake, river, or estuary. So, it's important to narrow the focus of the study so that the study is "do-able" while still answering the question of interest.

Here, for instance, it is unlikely that three samples would be enough replicates to characterize the whole lake well, and because of the patchy algal blooms, three samples probably would not be enough replicates to describe water quality conditions in the central area or the embayment, either.




Rivers are very patchy in physical, chemical, and biological conditions
pool – quieter, slower flow, lower nutrient supplies, easier for organisms to colonize (more abundant); more vulnerable to predators...
riffle – faster, more difficult to stay in the space; better nutrient supplies; predators have a harder time catching prey...
To answer a simple question – e.g., What is the ("true" or "real") average nitrate concentration? – requires accounting for this patchiness (MANY replicates), or narrowing the focus to consider only one type of habitat.


Carefully select the question…
Average animal density per unit riffle area?
Average abundance of 1 plant species?
In riffle areas only? In pools only?
Riffles
Pools

Characteristics / requirements of the target

Experiment: What is the impact of nutrient pollution (nitrate) on growth and survival of seagrass?

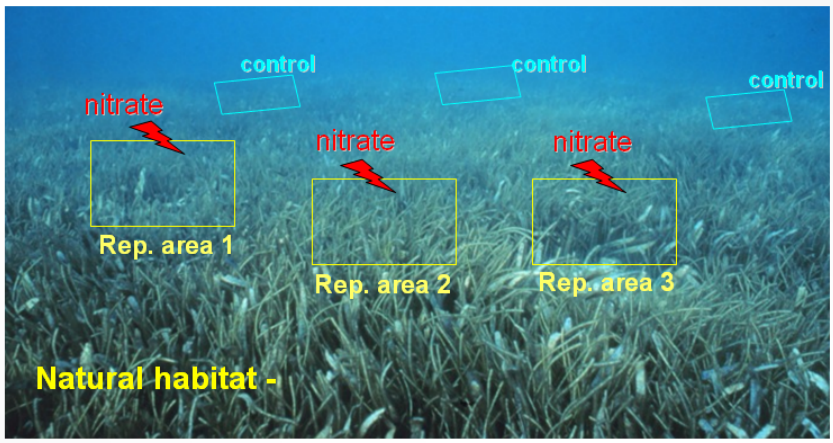
Possible approaches:

Natural habitat (difficult – very hard to keep all conditions except nitrate the same).

Mesocosms – moderately large containers (e.g. 1,200 liters). Lab.?: Indoor? outdoor? // Field?

Microcosms – small containers (e.g. flasks, small aquaria).

Natural habitats are often very difficult as places to conduct experiments. Consider this example -



Question

What is the impact of nitrate pollution on growth and survival of seagrass?

Consistent herbivore pressure (fish that eat the seagrass)?

Consistent depth (leads to different amount of available light)? [no]

Consistent effects of storm events? [no]

How much nitrate fertilizer will be needed with such enormous dilution potential?

Is it a good idea to add such pollution to the natural seagrass meadow?

How far away do the control areas have to be? Will the seagrass habitat be the same way over there?

Microcosms – small containers – are a lot easier to work with, although they have "container or wall effect" problems and it's hard to make them realistic, so again, it's important to design a question that can be answered well in such containers –

Mesocosms – medium-sized containers – are more realistic and have less "wall effects" -

Here, miniature seagrass beds were established in these mesocosms for experiments to assess the effects of nitrate pollution on plant growth and survival.