Decision Support Tool

**Step 3: Finalise the experimental design**

The [Decision Support Tool](https://meddle-scor149.org/decision-support-tool/) is a 3-step guide to help you plan your multiple driver research. Each step of the guide takes you through a different stage of the planning process: (1) define the research question, (2) identify responses, drivers and the design and **(3) finalise the design**. For more information see the complete resources for the *Best Practice Guide for Multiple Drivers Marine Research* on the [MEDDLE website](https://meddle-scor149.org/).

**Part 1: Response variables and/or traits of interest**

 ***Repeat for each response variable and/or trait of interest***

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| Have you chosen your treatment levels to reflect your best guess of expected response norms to the main drivers? See Figure 2 of [*Experimental strategies to assess the biological ramifications of multiple drivers of global ocean change - A review*](https://onlinelibrary.wiley.com/doi/full/10.1111/gcb.14102)):Does your design allow you to determine responses to both individual and multiple drivers? |  |
| What are the measurement units? * How will you standardize your response data (e.g. size, body weight, surface area, sex, time)?
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| What biological variation do you expect in the responses / traits?* Will this variation happen within samples, across time, across places (e.g. different populations, genotypes or phenotypes), or different states of acclimatization?
* Do you expect spatial and temporal dynamics in the responses or traits (e.g. latitudinal, diurnal or seasonal variation)?
* How can you accommodate this temporal and spatial variability in your measurements? Considerations include replication, time of measurement, sample origin, and analytical detection limit.
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**Part 2: Explanatory variables / drivers of interest:**

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| Are your drivers constant or variable? * Do you expect temporal dynamics or spatial variation in the drivers, e.g. tidal, diurnal, seasonal, weather dependent, or random fluctuations?
* How will you accommodate the variation in your measurements?
* For how long and over what spatial extent will you need to measure?
* What is the best sampling frequency and spatial resolution?
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| Are you interested in changes in the mean value(s) of your driver(s)? Or, variances, maxima or minima?* What are the measurement units for each driver?
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| For experiments, what is an environmentally relevant duration of exposure? * Is such a duration feasible?
* What are the constraints imposed by using shorter durations?
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| Which of your drivers are naturally confounded?* Can any drivers be omitted? For example, salinity and alkalinity typically co-vary very strongly. Temperature and aragonite saturation state are also related. Think about simplifying your design to drivers that do not co-vary strongly.
* Can you collapse some of the remaining drivers? These are typically not the primary driver of interest, and may co-vary in one of your treatment scenarios.
* Do you have the prerequisite knowledge to collapse these drivers, e.g.
	+ Do you know the dominant driver?
	+ What prerequisite knowledge necessary to answer your question?
	+ Will a collapsed design answer your research question (Step 1)?
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| How will you deal with physical-chemical interactions among drivers, e.g. salinity‒pH‒temperature, metals‒pH, pCO2‒pH‒DIC?   |  |

**Part 3: Experimental design and statistical analyses**

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| What is your sampling design? How many treatment levels per driver? * Do you have enough treatment levels for a regression analysis?
* Do you expect the response to be non-monotonic?

How many replicates per treatment level and control?* How variable are your replicates? Do you expect to see obvious or subtle effects?
* Do you have enough replicates for each level of a categorical analysis (ANOVA-style design)?
* What other drivers could introduce variability?
* Are there any covariates that could explain variability?

What is your experimental sampling unit / replicate? * This is typically the container or area to which you apply your experimental treatment. Is this the level you *want* to replicate? Are you *able* to apply each treatment independently at this level?
* Note: your sampling unit may be within each experimental unit (e.g. multiple fish in a tank), but if the treatment is applied to the tank, then the tank becomes the replicate.
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| Do you understand the relationship between the number of treatment levels, the number of replicates per level, and the sample units?Do you have preliminary data you could use for an *a priori* Power Analysis?Answering this question may be the most important component of your whole study! There are a range of resources that will allow you to run simulations and look at changes in the performance of your study:* [MEDDLE simulator](https://meddle-scor149.org/simulator/#introduction)
* [Dose-response simulator (R)](https://edild.github.io/lc50_bias_sim/)
* [G\*Power](http://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower.html)

NB these methods cannot be used for *post-hoc* Power Analysis. See the open access paper [*Experimental strategies to assess the biological ramifications of multiple drivers of global ocean change—A review*](https://onlinelibrary.wiley.com/doi/full/10.1111/gcb.14102) for more information. |  |
| What is the most relevant and valid control? * Is the concept of a “control” relevant? Would a gradient study be better (e.g. are you measuring gradients of change)?
* If using controls, are they locally or regionally relevant in all aspects (including co-limitations)?
* Have you considered using pre-industrial conditions as a main (or additional) ‘control’?

Do you also need a control from Time zero or a ‘baseline conditions’ control? * If you collected those data, what would you do with them?
* Do you need the same or a greater level of effort to collect this data point?
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| What type of data will your response and driver constitute? * Continuous variable, time-series measurements, point measurements, presence/ absence, count data, categorical data, compositional data…
* Univariate or multivariate responses?
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| What do you expect to be your greatest source of variation? * Is this variation biologically important, or is it due to experimental error, e.g. measurement error?
* How will you deal with this variability?
* Will you average measurements within a replicate, e.g. across individuals within tanks / sites?
* Are the levels of variation similar in all your treatments, or are the variances heterogeneous? If the latter, how will you deal with this when you analyse the data?
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| What type of statistical analysis or modelling approach will you use? What will be the specific statistical model you will apply to your data?Do you know how to implement this model and understand its results?* Does your model consider both individual main effects and interactions?
* Can it deal with both fixed and random factors (if present)?
* What is the power of your experimental design? Test your design with your simulated data set.
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| How can you validate your results?* Can you compare lab data against field data? Or use more than one measurement technique?
* Can you improve the quality assurance of the sample analyses using cross-lab comparisons or calibrations?
* What quality control processes will you use for the data?
* What criteria will you use for rejecting spurious data?

In theory, what could go wrong during the experiment, and how might this affect your data (e.g. can you separate biological contamination of a header tank from a treatment effect or are they confounded)? * Is this a plausible outcome? Can you redesign your study to address this?
* If you cannot modify your design for a plausible risk (e.g. due to physical constraints, biohazard risk, etc.), what can you do to help convince yourself and others that this outcome has or has not occurred?
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**Part 4: What resources do you need, and do you have access to all of them?**

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| For a **field study**, do you have access to:* Transport
* Personnel
* Sampling gear
* Measurement instruments and calibration kits
* How many days will you need to go into the field? How many contingency days will you need to account for bad weather?
* What infrastructure do you need to get your samples back to the lab (if relevant)?
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| For a **lab study**, do you have access to:* Laboratory / aquarium / seawater of appropriate quality
* Measurement instruments and calibration kits
* Biota
* Personnel for regular maintenance and measurement, as well as for final analysis and take-down.
* How long do you need to run the experiment for?
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| For **sample processing**, do you have access to: * Sample preservatives/storage
* Laboratory instruments
* Reagents
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| For **statistical analysis**, so you have access to:* Expert guidance, software
* Are you aware that statisticians or other experts are much more likely to provide useful help if you meet with them during the design stage rather than after data have been collected?
* Similarly, are you aware that statistical modelling cannot rescue a poorly designed study?
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| Time and costs: * How long will the whole study take, and much will it cost?

Try to upscale: How long will the measurements and sample processing take? How much will it cost to process each sample? Can I afford this? * Try upscaling with a spreadsheet:
1. List each individual step that needs to be done to execute the work
2. Add a column with your best guess of how long each step will take per sample.
3. Add another column with the cost per sample
4. Add another column for the number of samples.
5. Multiply columns (3) and (4) to estimate costs; multiply (2) and (4) to obtain the total amount of time. Remember to add time for breaks.
* Try to validate your estimates (we all are far too optimistic!). For example, ask a peer how many samples they have been able to process per day etc.
* If the numbers are too big, can you use alternate methods? For example, digital photographs of samples can be quicker than measuring directly *in situ*, but may be less accurate and require more time back in the office.
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| What are your most important constraints? * Time? Resources?
* Experience and skills of yourself and advisors, mentors and collaborators?

How can these constraints be overcome? For example, can you enter into collaboration with an additional person?  |  |
| If any of the constraints can’t be overcome, refine your question (Step 1 of the Decision Support Tool), and run through the three-step Tool again. |

**Part 5: Check your objectives again and see whether they are all met.**

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| Does this design really answer your main research question and objectives, which you outlined in first two steps of Step 1 of the Decision Support Tool? If not, is there scope to further fine-tune your question to make it more relevant, tangible or feasible?  |  |

