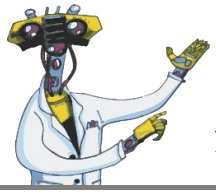

Experimental design for multi-driver studies

Tutorial: The Experimental Design video tutorial can be found on the MEDDLE for Multiple Drivers Research YouTube channel.

Speaker: Jon Havenhand, University of Gothenburg, Sweden

Video: Christina McGraw, University of Otago, New Zealand

Transcripts: Rebecca Zitoun, University of Otago, New Zealand

Resources: The complete resources for the *Best Practice Guide for Multiple Drivers Marine Research* are available on the MEDDLE website.

0:00 - *Introduction*

You probably know this already, but one of the most important things that we need to do when we are starting to design an experiment is to work out '*what are we going to include in our experiment?'* So we need an inventory list about all the drivers that might influence what we are interested in. And in case you haven't seen it already there is an excellent video as a part of this series by Philip Boyd that addresses '*how do you generate that inventory list and how do you begin to tease apart what you might be interested in*?'. So I recommend you look at that if you haven't seen it already.

But let's assume that I have already done that and I am interested in the impacts of pCO_2, which is going to go up with climate change. I am interested in the effects of oxygen, which is probably going to go down in the area where I work, in Scandinavia. And, I am interested in the effects of freshening, because the salinity is likely to go down there too. So I have got my three factors. I have got ambient and future pCO_2, I have got ambient and future oxygen, and I have got ambient and future salinity. So I have a 2-by-2-by-2 design, which is 2^3, that's 8 treatment combinations. It's the traditional, fully factorial ANOVA - I can do that.

But realistically, I know that there is a whole bunch of interesting things that are going to be going on between the pCO_2 of today and the pCO_2 of the future. I know that the response to oxygen is probably not going to be linear, and realistically I am not too sure about the response to salinity. So I might want to include more than just 2-levels of each one of those. And let's say for the sake of argument that I am really interested in pCO_2. So let's say I am going to pick 5 levels. I might pick pre-industrial, ambient, and then a value for 2100

perhaps, and maybe one in between, and one for the far future, so I have got 5 different levels of pCO_2.

So then I have got 5-levels of that. Realistically, if I am going to look at the response norm, if I want to get a decent curve out for how the response looks to different levels of oxygen or different levels of hypoxia actually, because the oxygen is going down, then I am going to need at least 3 points. So let's say that we are going to have 5 of those as well, and just for completeness sake we are going to have 5 salinities. Why not?!?. So we have got 5-by-5-by-5.

So suddenly my 2^3 experimental design has moved to 5^3 experimental treatment combinations, which is 125! And here is 125 treatment combinations all stuck together, 5-by-5-by-5. There are 125 bottles here, I figured that I might just, with the response variables that I am working with, I might just be able to measure everything that I need to measure in the time available using this design, but it's not even replicated. I only have one of each treatment combination here, so how on earth am I going to replicate this.

3:01 min – ***Option 1: Replicate in time***

One option is just to take this design and do it un-replicated this week. Now, my experiment is going to take me about 3 days – 4 days plus some set-up and take-down. So I can probably do one experiment in a week. So I can run this, this week.

Now, an important rider to all of this, we set this up so all of this looks like here is one treatment that is increasing, here is another treatment that is increasing, and here is another treatment that is increasing. The colours all match, you can see it really nice and clearly, that is the whole point.

I would never run the experiment this way. These bottles are all closest to the window, these ones are all closest to the corridor over there, these ones are in the shade, these ones are in the light, these ones are under my armpit - God knows what's going on there. You wouldn't do it this way, right? You would randomize all of these. So don't do it this way, but it's illustrative.

So I can do it this week, get my data, and then I do it again next week, get some more data. I will do it again the week after, get some more data. And that replication in time is going to introduce a bunch a variability into the experiment. But realistically, that is okay, because that variability just goes along with sampling error and normal noise, and contributes to the error term in our subsequent analysis. The advantage of replicating in time is that you can replicate it, within reason, as many times as you like. You can almost keep going until you have got enough statistical power to detect the effects that you want to detect.

4:27 min – ***Option 2: Major vectors***

So if we are not going to replicate in time, but we want to find some other way to run our experiment to reduce these 125 treatment combinations to something more manageable, then we can replicate within one time within the experiment. How do we do that?! One obvious option is to say: '*well realistically, am I interested in what happens in this bottle here? or in that treatment combination that is right there? Or is what I am really interested in, what happens in this primary vector here, and this primary vector here, and this primary vector there*?'. That is the 3 major factors, this is our pCO_2, our oxygen, and our salinity, and maybe the combination of those 3, the diagonal that goes through the middle of this cube that starts down here and ends up here. Because that is going to tell me the individual effects of salinity, the individual effects of oxygen, the individual effects of pCO_2, and the combined effects of those 3. It won't tell me the 2-way interactions, but realistically I might not be interested in that. So what might that look like?! Well, with the aid of my magic wand (5:40 min)...

So now we have the 3 primary vectors, the pCO_2, the oxygen, and the salinity, so we can work out what each of those is doing independently. But we only have the 3-way interaction between those, which is our projection into the future of the combined effects of pCO_2, oxygen, and salinity, progressively into the future. So this design gives us a scenario into the future, and some basic mechanistic understanding, but we are still lacking the 2-way interactions between these variables. So we lack some mechanistic understanding of this design.

6:30 min - ***Option 3: Scenarios***

If that last design was too big, then we can ask ourselves: '*if we are going to reduce this, and we don't want to know what's in each one of these different treatment combinations, what do we really want to know?'.* And that's really a question of: '*do we want this whole design or do we just want to know what's likely to happen for example in scenarios into the future?'.* Where this is today, this is our ambient condition, so this is today's pCO_2, today's oxygen, today's salinity, and this is a future, acidified, hypoxic, fresher world. So realistically, what we want to know is not even these major vectors and the diagonal, it's just the diagonal. It's the scenario. Yes, we confounded hypoxia, and salinity, and pCO_2, but if all we want to do is find out what is going to happen in the future ocean and we are pretty sure that that's what that future ocean is going to look like, then that's all we need, right?! And we can easily replicate that, because that's 1,2,3,4,5 different treatment combinations. So, with the aid of my magic wand again (7:40 min)…

So what we have left here is the 3-dimensional diagonal through the matrix. So we have the lowest value of pCO_2, the next highest value, the third highest, the fourth highest and so on, but these are confounded with the same equivalent values of oxygen and salinity. So we have a scenario. There is only 5 treatment combinations, we can easily replicate this and get a really good idea of how these co-varying drivers are going to influence our response into the future. But we know nothing from this design about how each of those drivers operate independently.

8:33 min - ***Option 4: Collapsed factorial***

Another way that we might be able to reduce the size of this design and thereby enable replication is to go back, and again ask ourselves the question '*what is it that we really want to know*?'. Now, if you remember right back at the beginning I said that I was really interested in the effects of pCO_2, and that's why I wanted 5 different levels of pCO_2 to get some good resolution on pCO_2. And then I got carried away and I went for 5-levels of oxygen and 5-levels of salinity and we end up with this problem that you can see before us.

But if what I am really interested in is the effect of pCO_2, then I could maybe keep my 5-levels of pCO_2 and then just collapse the other 2 variables, salinity and oxygen or hypoxia, into one variable. Let them co-vary. There could be more drivers in there as well, but I just got those extra 2 in this. So if we are going to generate one of those collapsed designs as they are called, which other people have used - Philip Boyd and his co-workers have published on this (Nature Climate Change volume 6, pages 207–213 (2016)) and you can find the reference on the website -, what would one of those collapsed designs look like if we are going to turn this into a collapsed design. So, with the aid of my magic wand once again (9:43 min)…

Okay, so if we want to reduce this even further, we can ask ourselves the question once again '*what's the primary driver that we are interested in here?*'. And in this case, it's pCO_2, it's this vertical aspect. So we are going to collapse the other 2 drivers in this case. We have got oxygen in this direction and salinity in this direction. So we are going to use all 5 values of pCO_2 at ambient oxygen and salinity, and all 5 values of pCO_2 at future oxygen and salinity. I am just going to get rid of everything else. So now we have all 5 values of pCO_2 at ambient oxygen and salinity, and all 5 values of pCO_2 at future oxygen and salinity, and we have lost everything else from the design. Here we have got 10 treatment combinations. We have retained our resolution in terms of pCO_2, and we lost everything else to do with the co-variance between oxygen and salinity. But if you want to simplify this even more, particularly if you need more replication, 10 treatment combinations is quite a lot. We can cut this down to 4 by removing these middle 3 pCO_2's, so that we have ambient pCO_2 and future pCO_2, ambient and future at each of, ambient oxygen and salinity, and future oxygen and salinity. So let's go ahead and do that (11:19 min)…

And now we have that collapsed design that Boyd et al. used. We have ambient oxygen and salinity at ambient pCO_2 and future pCO_2, and future oxygen and salinity at ambient pCO_2 and future pCO_2.

The Boyd et al. example collapsed more drivers together than just oxygen and salinity, but the design is essentially the same. We have 4 treatment combinations and we can do lots of replication with this.

11:46 min - ***Summary***

So now you have got some options on how we might be able to deal with either replicating our grand design with 5-levels of pCO_2, 5-levels of oxygen, 5-levels of salinity, or whatever drivers you might be interested in. You might be able to replicate that in time or you might not be able to replicate that in time. But realistically, before you decide which one of those you want to do, you need to ask yourself again the question '*Why do I want to know? What is it that I really want to understand?*'.

If I am interested in the mechanics of how pCO_2, and oxygen, and salinity influence the response variable in my system, and I really want to understand all the nuances in there, then maybe this design, this full 125 treatment combinations design, is what I need to do. And then I am going to have to replicate it in time, or maybe get a better grant, or persuade all my colleagues to come and help me run it 3 times and the same time – I don't know, whatever your solution might be. You might, depending on the system, be able to replicate this in space at the same time, but it is pretty unlikely. You have got to be able to measure all of these containers.

So that's one option, and that's probably, realistically the only option, if you really do want to know absolutely everything about the different combinations within here. But there are other options too. We have seen, for example, the major vectors, which just look at the primary drivers in the system. The 3 primary axis in this cube, and the diagonal combination of those. So that will tell us some mechanistic information. It will tell us what each one of those 3 drivers, pCO_2, oxygen, and salinity, are doing in our system, and what the combination of those into the future will do. So it tells us something that's sort of like a half-way house between the mechanistic option and the next option, which is just to look at the diagonal, all the way from down at ambient to future.

We just looked at that 3-D diagonal vector. That's simply looking at a future scenario. So that's putting our oxygen, our pCO_2, and our salinity together. They are co-varying at values that are projected for the future, and we don't know which one of those is driving the response. But that might not be interesting, if what we want to do is inform policy. If we want to say '*so how is the future ocean going to influence the respiration rate or whatever it might be?*', then we can do that with a design like that. It has disadvantages, because we don't know everything else that is going on. But we have only got 5 treatment combinations in there, so we can replicate that really well and get a lot of statistical power.

And then lastly there is slightly more complex designs like the collapsed design that we showed you, where you take the driver that you are really interested in, pCO_2, and you collapse the other 2. And just do a 2-way design, where the other factor is actually a combined vector of, in our case salinity and oxygen, but it could have other drivers in there as well. Again you confound those extra drivers, you don't know which one of those is working, but if you are really interested in the effects of, in our case pCO_2, then that's probably going to tell you what you need to know.

14:47 - *Pros and cons*

So pros and cons. Now, each one of those different designs has got different analysis options, and has different implications for how much replication you need, and how easy it is to get out information. Each of them also has implications for what you are not going to find out, so you need to think hard about what the designs are not going to tell you as well. I am not going to deal with either of those, but Peter Dillingham has got an excellent video on how you take the next step and look at analysing these different kinds of designs. I recommend you watch that.