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Report on design and schedules of experiments and field studies
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1. (Popular) Description of deliverable

This report describes the work carried out by researchers in workpackage 2 (Quantification of Impacts and Feedbacks) to prepare and perform experiments and field studies. The first mesocosm and laboratory experiments as part of the OceanCertain project will be performed in Patagonia, Chile (MESO-PAT, 22 October to 24 November 2014) at the Huinay Field station in the Commune of Hualaihué. The station lies between the Comau fjord in the Province of Palena and the border with the Republic of Argentina. Its location (42°22´ S, 72°24´ W) corresponds to the biogeographical Province of Magallanes and specifically to a zone that is highly representative of the continental fjords of North Patagonia.

Established in 1998 by Endesa Chile and the Pontifica Universidad Católica de Valparaiso, the San Ignacio del Huinay Foundation was created with the purpose of facilitating ongoing scientific research as well as seeking to preserve the biogeographical heritage of Huinay by means scientific research and sustainable development techniques. The Huinay Scientific Field Station was inaugurated on December 3, 2001 and has commenced facilitating pioneering research in a variety of fields. Today this is the only scientific field station in all the vast Chilean Patagonia. The station is well equipped with state of the art laboratories, small boats, meeting rooms, and dormitories for visiting scientists.

This report describes the status of the planning and preparation of the mesocosm and laboratory experiments planned at the Huinay station.

2. Summary of contribution of involved partners to deliverable

General set up:
The experiment a t Huinay will consist of two parts. First we are going to incubate fjord water in 10 large cubic PE containers with a volume of $1m^3$ each (mesocosms) on a floating plastic jetty. In this part of the experiment we will investigate the combined effect of two different grazing pressures in combination with different concentrations of dissolved organic matter (DOM (glucose), see figure 1) on phytoplankton community composition and productivity.
After two weeks of incubation, we will take water from 4 mesocosms (marked in red in figure 1) and transfer it to cleaned 20 l collapsible plastic containers (microcosms) for further incubation in temperature controlled laboratories at the Huinay station. In these microcosms we will manipulate the nitrate to phosphate ratio (N/P), pH, and temperature (or C-DOM, see below) as shown in table 2 and 3.
For both the mesocosm and the microcosm experiment it is essential that we prevent and contamination with trace metals. The essential trace metal concentrations in the Comau fjord are low and we therefore expect that a contamination with e.g. iron could have significant impacts on phytoplankton productivity and thus affect the outcome of this project. Also as part of this experiment we will investigate the effect of zooplankton grazing, temperature, and pH on the bioavailability of the essential trace metal iron and any iron contamination could falsify our results. We will therefore be extremely careful to prevent contamination by cleaning the mesocosms and microcosms with soap and diluted acid solutions. To avoid contamination during sampling, the containers will be moored in the fjord at a custom built floating plastic jetty which will be finished in August 2014. Both, the mesocosms and

microcosms will stay closed during the whole incubation period and sampling will be done by pumping water though cleaned hoses. Sample handling will only be done in laminar flow benches, which are designated for trace metal work. In total we will have 5 people on site who are trained in trace metal clean working procedures and who will be responsible for cleaning and sampling.

Mesocosm experiment:
The containers will be custom built in Chile and directly delivered to the Huinay station. During the experiment we will guarantee regular mixing of the water within the containers by rotating them manually on the side of the floating plastic jetty (figure 1 and 2). Grazing pressure will be adjusted by removal of zooplankton instead of addition of extra zooplankton. This is because sampling and picking of zooplankton is very time consuming and extremely stressful for the animal and usually results in higher mortality rates.
The mesocosm will be performed in two weeks of the experiment period then water from 4 mesocosms will be transferred to the bottles for lab experiments.

Microcosms experiment:
For these incubations we will use 20 l collapsible PE containers. Water will be taken from these 4 mesocosms: 10 (low grazing pressure, background DOM concentration), 6 (low grazing pressure, medium DOM concentrations), 5 (background grazing pressure, medium DOM concentrations), and 9 (background grazing pressure, background DOM concentrations). We will not chose the mesocosms with the highest DOM concentrations added as could represent unnatural starting conditions.
The collapsible containers have the advantage that they collide when water is removed and therefore you don't have to introduce a headspace. This is essential as we want to keep the pH level I these experiments constant and a headspace with laboratory air could affect the pH in the seawater. The different stressors in this set of experiments will be N/P ratio, pH, and C-DOM or temperature. At the moment it is still open for discussion whether we will use DOM or temperature as an additional stressor depending on the development of the mesocosm experiment.
We are also still discussing whether we will have an experimental design with 96 bottles in total (see table 2) or 64 bottles in total (see table 3). The final decision of the experimental set up will be made on site depending on the development of the first experiment.
We are going to use RCP 8.5 (Representative Concentration Pathway) estimation for temperature anomaly (pCO_3 = 1370 ppm (for low pH), N:P(:Si:Fe) ratio will be decided according to local conditions). Final concentration of high CDOM is open to discussion and will be decided according to local conditions.

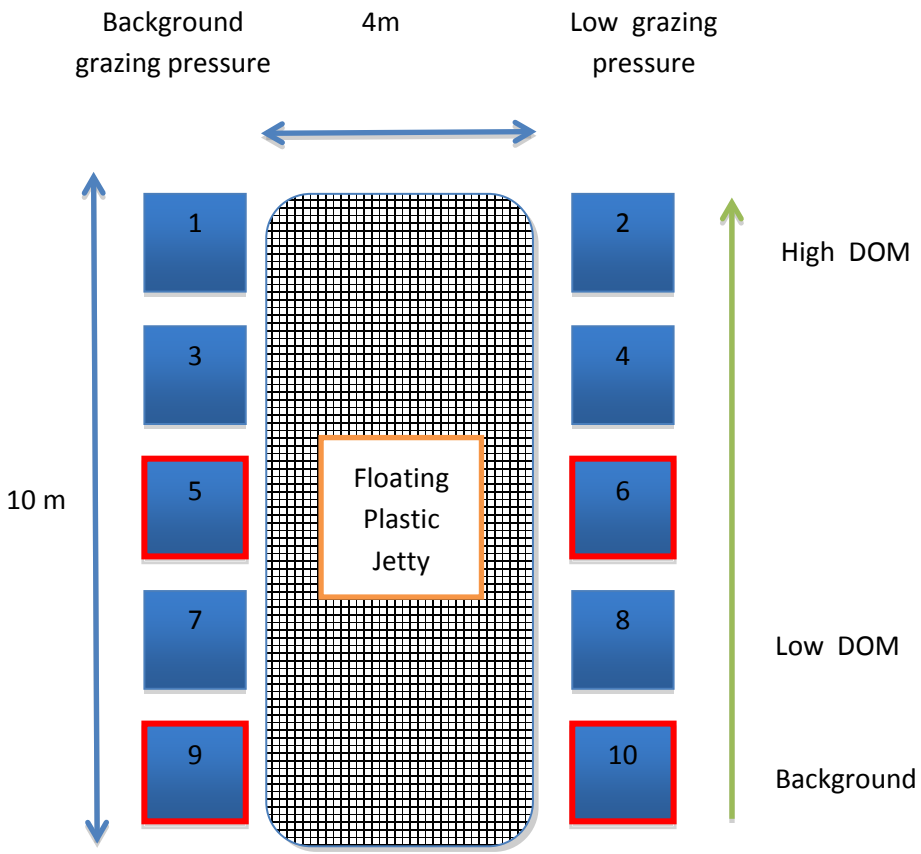

Fig.1: mesocosm set up

Table 1: Parameters analysed and responsible partners during the mesocosm and microcosm experiments:

Parameter	Responsible group	Method used
Temperature	UACH	Sensor HOBO
Light	UACH	Sensor, CTD (HG)
In vivo fluorescence	UACH (HG)	Fuorometer
Macronutrient concentrations	UACH, PUCV, CIEP	Manual or Autoanalyser
Trace metal concentrations (total and dissolved)	NTNU	FIA
Trace metal ligands	GEOMAR	Voltammetry
Carbonate system (DIC) and pH	CIEP	Sensor (CIEP)
Total and fractionated chlorophyll concentrations	UACH (HG)	Fluorometer
Total organic carbon (TOC), dissolved organic carbon (DOC), particulate organic carbon (POC), particulate organic nitrogen (PON)	UACH, CIEP, USA	C/N elemental analyser
Cell counts of viruses, bacteria, Cyanobacteria and eukaryotic phytoplankton	NTNU	Flow Cytometry
Microzooplankton and larger phytoplankton	UACH	microscopy
Bacterial production	UACH	To be clarified
Coloured dissolved organic matter (cDOM)	GEOMAR	Spectrophotometry
Phytoplankton pigments		HPLC
Primary production	UiB	BOD bottles + Oxy-mini optode

Tentative time schedule:

21st October:
All participants will arrive Puerto Montt

22nd October
About 11:00 transportation to Horno Piren by minibuses ca. 2 hrs
From Horno Piren to Huinay, by boat –ca 3 hrs
Arrival to Huinay research station about 16:00-17:00

23rd – 27nd October
Preparations, cleaning

28th October
START for Mesocosm experiment

10th November
FINISH for Mesocosm exp. & water transfer to the microcosms

11th November,
START bottle experiment

18th November
FINISH the bottle experiment

19th-22nd November
Packing, cleaning

23rd November
Transport to Puerto Montt

24th November
Official end of the MESO-PAT in Puerto Montt

Table 2: Option 1: Planned experimental set up for the microcosms with three different N/P ratios. The total number of bottles will be 96. If total manpower will be 20 persons, 5 persons will be responsible for each set of treatments (i.e. 24 bottles). If we allocate 25 persons for mesocosm experiment, then 6 persons will be responsible for each set of the treatments.

COMBINATION	1	2	3	4	5	6	7	8	9	10	11	12	
	N/P-1	N/P-1	N/P-1	N/P-1	N/P-2	N/P-2	N/P-2	N/P-2	N/P-3	N/P-3	N/P-3	N/P-3	
	pH-1	pH-1	**pH- 2**	**pH- 2**	pH-1	pH-1	**pH- 2**	**pH- 2**	pH-1	pH-1	**pH- 2**	**pH- 2**	
	No CDOM	High CDOM	No CDOM	High CDOM	No CDOM	High CDOM	No CDOM	High CDOM	No CDOM	High CDOM	No CDOM	High CDOM	
# Bottles													**Taken from cubitainer #**
12	Com 1	Com 2	Com 3	Com 4	Com 5	Com 6	Com 7	Com 8	Com 9	Com 10	Com 11	Com 12	
12	Com 1	Com 2	Com 3	Com 4	Com 5	Com 6	Com 7	Com 8	Com 9	Com 10	Com 11	Com 12	**10**
12	Com 1	Com 2	Com 3	Com 4	Com 5	Com 6	Com 7	Com 8	Com 9	Com 10	Com 11	Com 12	
12	Com 1	Com 2	Com 3	Com 4	Com 5	Com 6	Com 7	Com 8	Com 9	Com 10	Com 11	Com 12	**6**
12	Com 1	Com 2	Com 3	Com 4	Com 5	Com 6	Com 7	Com 8	Com 9	Com 10	Com 11	Com 12	
12	Com 1	Com 2	Com 3	Com 4	Com 5	Com 6	Com 7	Com 8	Com 9	Com 10	Com 11	Com 12	**5**
12	Com 1	Com 2	Com 3	Com 4	Com 5	Com 6	Com 7	Com 8	Com 9	Com 10	Com 11	Com 12	
12	Com 1	Com 2	Com 3	Com 4	Com 5	Com 6	Com 7	Com 8	Com 9	Com 10	Com 11	Com 12	**9**

Table 3: Option 2: Planned experimental set up for the microcosms with two different N/P ratios. The total number of bottles will be 64. If total manpower will be 24 persons, 6 persons will be responsible for each set of treatments (i.e. 16 bottles).

COMBINATION	1	2	3	4	5	6	7	8
	N/P-1	N/P-1	N/P1	N/P-1	N/P2	N/P-2	N/P2	N/P-2
	pH-1	pH-1	**pH-2**	**pH-2**	pH-1	pH-1	**pH-2**	**pH-2**
	Temp-1	Temp- 2	Temp-1	Temp-2	Temp-1	Temp- 2	Temp-1	Temp-2

# Bottles									**Taken from cubitainer #**
8	Com 1	Com 2	Com 3	Com 4	Com 5	Com 6	Com 7	Com 8	**10**
8	Com 1	Com 2	Com 3	Com 4	Com 5	Com 6	Com 7	Com 8	
8	Com 1	Com 2	Com 3	Com 4	Com 5	Com 6	Com 7	Com 8	**6**
8	Com 1	Com 2	Com 3	Com 4	Com 5	Com 6	Com 7	Com 8	
8	Com 1	Com 2	Com 3	Com 4	Com 5	Com 6	Com 7	Com 8	**5**
8	Com 1	Com 2	Com 3	Com 4	Com 5	Com 6	Com 7	Com 8	
8	Com 1	Com 2	Com 3	Com 4	Com 5	Com 6	Com 7	Com 8	**9**
8	Com 1	Com 2	Com 3	Com 4	Com 5	Com 6	Com 7	Com 8	

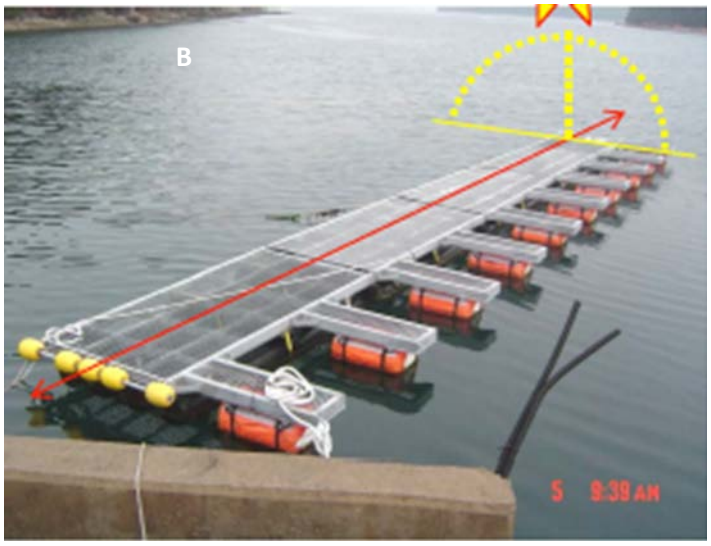

Figure 2: planned floating system for MESO-PAT in October/November 2014.

Figure 3: Incubations during the WAFOW 1 and Fe-DOM experiments. Here 35 litre bottles have been used in situ incubations at Huinay.

Fig. 3 . Floating 1 m^3 mesocosms during the WAFOW II experiment at Huinay in 2011.