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Abstract	This Deliverable describes the detailed plan for Joint Research Activities (JRA) demonstrating the ability of the AQUACOSM consortium to perform internationally coordinated standardized experiments to investigate effects of specific global drivers across the wide range of climatic and salinity zones in Europe. The effect of brownification on aquatic ecosystems is used as a pilot-example. The objectives, hypotheses and relevance of the coordinated experiments are elaborated. Additionally, methodology, technical approaches and measurements to be conducted are summarized and explained.
Keywords	DOC, mesocosm, plankton, global change, freshwater, marine, joint experiments

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1. Executive summary

Planned experiments in WP9 (Joint mesocosm experiments - JOMEX) will demonstrate the ability of the consortium to perform joint directed experiments along gradients from the Arctic to the Mediterranean and from freshwater to full marine systems. Such joint experiments will allow disentangling site-specific effects from general effects of experimental manipulations and thereby increase its predictive power. The strategy for JOMEX includes five experimental sites covering Arctic, temperate and Mediterranean waters and freshwater, brackish and full marine systems. The scientific approach is investigating the systems responses to dissolved organic carbon (DOC) pulses from terrestrial sources. The increasing transport of matter from terrestrial environments into aquatic systems is a highly relevant aspect of global change; its consequences for ecosystem dynamics are still poorly understood. The response to the pulse should depend on the composition of plankton communities and their history to the exposure to terrestrial DOC pulses in terms of frequency and strength. We would therefore expect strong site-specific differences in the response to a defined DOC pulse. To investigate the effect sizes of site-specific and general responses we will install nine mesocosms at each site, three controls, three treatments including a highly standardized DOC source and three treatments including a local, more site-specific DOC source. We will follow the responses of bacteria, phytoplankton and zooplankton to the DOC pulses and analyse amongst others the resistance, resilience and recovery of the communities. We expect that these experiments will clearly demonstrate the surplus value of the available infrastructures of AQUACOSM in terms of joint investigations of urgent research questions such as for example how global change related stressors affect European waters. The experiments will therefore act as a base for future joint research activities beyond month 48 of AQUACOSM.

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2. Joint Mesocosm Experiments (JOMEX) – Strategy

2.1 General objectives:

The joint experiments in WP9 (JOMEX) will demonstrate 1) the ability of the consortium to plan and conduct mesocosm experiments in European waters, from freshwater to full marine systems and from Arctic to Mediterranean environments. 2) The ability of the consortium to plan and conduct mesocosm experiments also at sites without permanent consortium infrastructure. 3) The surplus value of joint experiments by allowing to disentangle site-specific from general effects of experimental manipulations.

2.2 Sites:

- Freshwater system: Denmark (responsible partner: AU)
- Brackish system: Finland (responsible partners: UH, SYKE)
- Full marine system: Norway (responsible partner: LMU)
- Arctic system: Norway (responsible partners: UIB, UNI)
- Mediterranean system: Greece (responsible partner: HMCR)

All experiments will be installed and performed by the groups responsible for the specific sub-tasks.

2.3 Timetable:

- Freshwater experiment: August 2019
- Brackish experiment: June/July 2019
- Full marine experiment: August 2019
- Arctic experiment: Summer 2020 (exact month depending on local permits)
- Mediterranean experiment: June 2020

2.4 General design of mesocosms:

We will install nine mesocosms at each experimental site; mesocosms will have a volume of about 2100 L, a depth of 3 m and a diameter of 1.1 m. These mesocosm dimensions are well established in marine and freshwater field experiments allowing maintaining phyto- and zooplankton populations over several weeks. An estimated daily maximum sampling volume of 15 L will result in less than 10 % volume loss during three weeks of experimental duration. The mesocosms have a conical bottom, an opaque outside, and a top cover. Mesocosms will have a ring around the top to fix the mesocosms to floating rafts (Fig. 1).

Fig. 1: Floating raft design for WP9 experiments.

2.5 Filling of mesocosms:

Mesocosms will be filled by either pumping water into bags or lifting bags from a certain water depth to the surface. The choice of method will depend on site-specific characteristics such as available energy supply to run pumps, the need to use a boat for transporting water to the mesocosm installation sites and maximum water depth at the incubation site. Great care will be taken to fill control and treatment bags with exactly the same method and at the same time to ensure a maximum initial community similarity between all mesocosm bags.

2.6 Experimental hypotheses:

We will investigate the response of different plankton communities to a single DOC pulse and will follow the reaction of the system after this strong disturbance in terms of resistance, resilience and recovery. We hypothesize that 1) systems, which experience regular disturbances by DOC pulses are more resistant to such a pulse and 2) systems with high initial functional diversity will have a faster recovery towards initial conditions.

2.7 Experimental manipulation & design:

We will perform a pulse experiment with DOC, the concentration will be 2 mg/L. Treatments will include three control mesocosms without DOC addition, three experimental treatments with a highly standardized DOC form (HuminFeed; HUMINTECH, Grevenbroich, Germany) and three experimental treatments with a local DOC source. The experimental duration will be a minimum of two weeks at all sites.

2.8 Measurements:

We will follow the transient dynamics of a plankton community after a DOC pulse. Bacterial, phytoplankton and zooplankton parameters in terms of community function and community composition will be measured. We will characterize the resistance of the different communities to the DOC pulse and hypothesize systematic differences. Measurements that are central to test the hypotheses will be performed at all sites in comparable ways; additional measurements (extended parameter list) will be performed (supported by other funds and cooperation between partners) and will allow site-specific further insights into experimental dynamics. All measurements will follow described SOP procedures assembled within AQUACOSM (https://www.aquacosm.eu/download/deliverables), for measurements were SOPs not necessarily exist relevant references are given.

2.8.1 Abiotic environment measurements

- Temperature: daily
- pH: daily
- Oxygen: daily
- Conductivity: daily
- Light (µmol PAR): daily
- Extended parameter list:
    - Spectral light analyses (Spectroradiometer)

2.8.2 Nutrients, total & particular organic carbon (TOC, POC), particular organic nitrogen (PON) measurements:

- Total phosphorus & dissolved inorganic phosphorus (TP, DIP): twice a week
- Nitrate (NO_3), nitrite (NO_2), ammonium(NH_4): twice a week
- Particulate organic nitrogen (PON): twice a week
- Silicate (Si): twice a week
- Particulate organic carbon and total organic carbon (POC, TOC): twice a week

2.8.3 Bacterial parameter measurements

- Bacterial abundance (fluorescence based methods: FlowCytometer/ fluorescence microscope): twice a week
- Extended parameter list:
  - Bacterial community structure (16SrRNA)
  - Bacterial production

2.8.4 Phytoplankton parameter measurements

- Phytoplankton chlorophyll *a* (extracted): daily
- Phytoplankton community structure (Lugol fixed samples for microscopy): at start, middle and end day.
- Extended parameter list:
  - FlowCytometry counts
  - Chlorophyll *a* fractionated (0.2, 2, 10 µm filter size)
  - Phytoplankton community structure (16SrRNA)
  - Pigment composition of phytoplankton (HPLC, Wright et al. 1991)
  - Efficiency of Photosystem II (PAM analyses, LIT)
  - Mixotrophy (LysoTracker, Anderson et al. 2017)
  - Size distribution (Cell counter)
  - Nutrient limitation assays (Andersen et al. 2007)

2.8.5 Zooplankton measurements

Microscopic analyses of microzooplankton (Lugol samples): start, middle and end day

Mesozooplankton samples (> 250µm), microscopic analyses of abundance and community composition: start and end day

2.9 Data handling

Handling of data will follow the general data strategy described in WP 4.2 (http://aquacosm.eu/download/deliverables/D4.2%20Database%20management%20Plan_final.pdf). Data will be stored at local servers of partners performing experiments and at central AQUACOSM data storage structures.

2.10 Analyses

Experiments are short-term single pulse disturbance experiments. It is therefore possible to extract and analyze four important response measures (a-d, Fig. 2a) characterizing the transient dynamics of the systems after single pulse disturbance and allowing to test the general hypotheses. Measures will mainly be made for community function stability (for example Chl *a*) and where possible also for community composition stability.

a) Resistance to disturbance: ability to withstand the perturbation at the start of the experiment
b) Resilience: recovery trend after the perturbation
c) Recovery: degree of functional or compositional restoration at the end of experiments
d) Temporal stability: residuals around the resilience slope

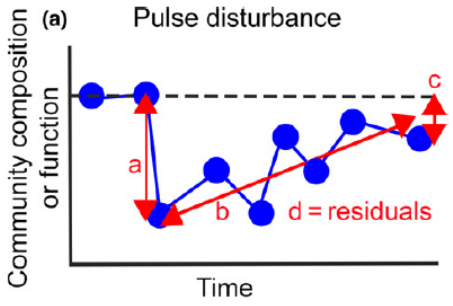

Fig. 2: Graphical (2a) and mathematical (Table 1) definitions of the transient dynamic measures (adapted from Hillebrand et al. 2018)

Table 1 Stability measures addressed in this study, with mathematical definition and interpretation

Measure	Timing	Function *F*	Interpretation	Composition *C*	Interpretation
a: Resistance	Initial sampling after disturbance	Measured as initial log response ratio $a = \ln\left(\frac{F_{dist}}{F_{con}}\right)$	Benchmark: 0 = maximum resistance if dist = con $a < 0$ = low resistance through underperfomance a > 0 = low resitance through overperfomance	Measured as initial similarity $a = \mathrm{sim}\left(\frac{C_{dist}}{C_{con}}\right)$	Fixed range 0–1. Benchmark: 1 = maximum resistance as dist = con $a < 1$ = low resistance
b: Resilience	Intermediate samplings	Measured as slope of regression of relative function over time: $\ln\left(\frac{F_{dist}}{F_{con}}\right) = i + b * t$, where i = intercept, t = time	Benchmark: 0 = threshold, indicating no recovery $b > 0$ (more rapid) recovery; $b < 0$ = further deviation from control[1]	Measured as slope of regression of similarity over time: $\mathrm{sim}\left(\frac{C_{dist}}{C_{con}}\right) = i + b * t$, where i = intercept, t = time	As for function
c: Recovery	Final sampling	Measured as final log response ratio $c = \ln\left(\frac{F_{dist}}{F_{con}}\right)$	Benchmark: 0 = maximum recovery where dist = con c < 0 incomplete recovery c > 0 overcompensation	Measured as final similarity $c = \mathrm{sim}\left(\frac{C_{dist}}{C_{con}}\right)$	Fixed range 0–1. Benchmark: 1 = maximum recovery when dist = con; $c < 1$ incomplete recovery
d: temporal stability	Intermediate samplings	Measured as inverse standard deviation of residual around resilience (see b) $d = \left(\frac{1}{\mathrm{sd}(\mathrm{resid}_b)}\right)$	No benchmark, larger d corresponds to lower fluctuations around trend	Measured as inverse standard deviation of residual around resilience (see b) $d = \left(\frac{1}{\mathrm{sd}(\mathrm{resid}_b)}\right)$	As for function

The table specifies the following information for each measure: when it is measured (Timing), how it is measured and how it can be interpreted (Interpretation). Measurements are given separately for functional (F) and compositional (C) stability. The letters a–d correspond to Fig. 1, dist are the disturbed treatments, con marks the control. Benchmark values and ranges are given if possible, benchmarks being either a maximum or a threshold value.
[1]If the response to disturbance is not a decrease but an increase in the function (i.e. i = positive), then a more negative slope corresponds to a faster recovery (i.e. higher resilience).

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Beside the above-described analyses of community responses to the pulse disturbances, which will be done at all sites, more site- and species-specific analyses, will be performed. These will be dependent on the initial experimental community species composition and the responses to the disturbance. All responsible partners have large experience in analyzing such type of experiments. By comparing the responses of communities at all sites to the DOC pulses in terms of above described stability dimensions we will be able to identify site-specific responses but also see general response patterns common to all sites.

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3. Dissemination activities related with the Deliverable

3.1 Dissemination strategy

After finishing an experiment a short report will be send to the project coordination and will be included in Deliverables 9.3, 9.4, and 9.5. Main available results of the experiments, including joint analyses of results confronting the initial hypotheses will be included in D.9.6 that will focus on strategies for further JRA projects. We plan to write at least one peer-reviewed publication including results from all five experiments and several additional publications reporting site-specific results.

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4. Appendix

None for this report.

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