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TRANSFERRING GRAND CHALLENGES INTO EXPERIMENTAL DESIGNS

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1. Abstract

Transferring Grand Challenges into experimental designs is important to get a causal understanding of the underlying mechanistic relationships how Grand Challenges affect ecosystem services. Additionally, experiments are needed testing Grand Challenges related mitigation strategies under semi-natural conditions. From discussions within WP6 of AQUACOSM-plus it emerged that mesocosm experiments dealing with Grand Challenges in aquatic environments can especially benefit from following design considerations: 1) Mobilization of mesocosm infrastructures; 2) Joint experimentation; 3) Automatization of high frequency measurements; 4) Up-scaling complexity. Within AQUACOSM-plus several work packages already act on these aspects. Hence, upcoming planning for large experiments dealing with Grand Challenges in 2022 and 2023 in AQUACOSM-plus can take above mentioned suggestions to transfer Grand Challenges into experimental designs already into account.

2. Experimental strategies for investigating Grand Challenges

Grand Challenges are human induced environmental shifts that pose risks to ecosystem functioning and thereby human well-being. Such shifts affecting European & global aquatic systems include rising global temperatures, increasing enrichments with nutrients, salinization of freshwater systems and the drastic loss of biodiversity to name just the most prominent examples. Assessing the environmental and social risks related to such Grand Challenges needs a detailed, concept-based, understanding of how Grand Challenges affect ecosystem dynamics and thereby ecosystem services. Such understanding may be important to support political activities to reduce/eliminate drivers of such unfavourable ecosystem changes. Additionally, it is important for developing potential mitigation strategies to face challenges where reducing the impact of harmful drivers might not be sufficient any longer to prevent negative consequences of on-going environmental shifts. Such strategies include so called "Nature-based solutions" inspired and supported by nature to benefit biodiversity and support the delivery of ecosystem services. Hence, such potential mitigation strategies need experimental proof of concept analyses on how to reduce such negative consequences by human intervention. Both aspects, a concept-based understanding and the testing of mitigation strategies to deal with Grand Challenges threatening aquatic ecosystems will have to include mesocosm experimentation. Mesocosm studies involving replication of treatments allow testing the effects of single or multiple factors on complex ecological communities, effectively identifying mechanistic relationships under semi-natural conditions. These conditions are not met from analysis of long-term data, nor from experimental analysis of focal taxa.

We identified four major design strategies, which have large potential impact to optimize mesocosm experiments related to Grand Challenges such as discussed within the Grand Challenges Webinars in AQUACOSM-plus, WP6. These technical aspects of experimental designs may alone, or in combination, improve the impact of mesocosm research on meeting Grand Challenges.

The four strategies were:

- 1) Mobilization of mesocosm facilities/setups
- 2) Automatization of measurements and generation of HF data within mesocosms
- 3) Joint mesocosm experimentation
- 4) Up-scaling of mesocosm experiments

2.1 Mobilization of mesocosm systems:

Mobilization of experimental systems is an important aspect in investigating Grand Challenges. Consequences of Grand Challenges are typically context dependent, resulting in spatial heterogeneity in terms of sensitivity of ecosystem responses to e.g. eutrophication or salinization. Investigations of such "regional hotspots" of Grand Challenges need transportation of mesocosm systems when fixed mesocosm systems are not available at the most relevant sites. Hence, mobilization implements a modular and demountable design of mesocosms. Within the projects AQUACOSM and AQUACOSM-plus two different demountable designs have been newly developed. First, within AQUACOSM a large system for coastal and wave exposed environments was developed at Umeå University, Sweden (UMU, WP7, D7.4), that even can withstand ice cover for extended period of times. Second, within AQUACOSM-plus a lightweight and therefore highly mobile system for lakes and protected areas that allows to study benthic-pelagic coupling is under development at Ludwig-Maximilians-Universität München, Germany (LMU, WP7). Third, large and seagoing mesocosm systems for ship based mesocosm operations are already present within the consortium (KOSMOS, GEOMAR Kiel).

The three systems bridge a full range of mesocosm systems necessary to investigate most pelagic or benthic-pelagic systems. They are: **1)** Cost effective lightweight systems that can easily be brought to and deployed in virtually any lake or protected coastal system, allowing high replication of treatments; a transport by van or truck is easily possible. Investigations with these mesocosm systems are possible all over Europe (and beyond), also on low research budgets. **2)** Larger, coastal systems, that allow mesocosm experiments in wave exposed environments or during winter ice conditions where experimentation in situ was not possible until now and only indoor simulations are performed. However, especially ecosystem dynamics during winter conditions and under ice may include important information related to seasonal dynamics in aquatic ecosystems influenced by global change. These robust systems need transport by standard ISO containers which can be handled by most freight forwarding companies on a routine base. **3)** The large seagoing mesocosm systems are demountable but pose more demanding requirements in terms of handling and transport and need support by a research vessel for operation. These systems allow investigating ocean (or great lakes) related challenges on a global scale and their large size allows enclosing complex communities and dynamics.

In summary, available mobile systems within the AQUACOSM-plus consortium will allow to investigate Grand Challenges at all potential European sites of interest, independent of whether a permanent mesocosm infrastructure is available at these sites.

2.2 Automatization of high frequency measurements within mesocosms:

Grand Challenges (Global change, salinization, eutrophication, acidification) are often associated with directed shifts in abiotic environmental parameters (temperature, pH, conductivity). Methods and infrastructures to quantify these parameters are therefore central aspects of such experiments. Beside abiotic parameters, also standing stocks of organisms and related state parameters (e.g. O_2 saturation, chlorophyll *a* concentration, turbidity) affected by biotic processes (primary production, community respiration) have to be analysed and monitored. Measurements of state parameters represent proxies for important trophic groups and are increasingly acquired by sensors such as fluorescence sensors for chlorophyll as a proxy for phytoplankton biomass, measurements of conductivity and the bicarbonate system, as well as image analyses systems such as for zooplankton. Sensors allowing measuring the photosystem activity of algae and oxygen analyses give important data for estimating primary production, respiration and carbon budgets

Such analyses are often taken in a manual way, either by suspending probes and doing individual point measurements or by taking water samples into laboratories for further analyses, usually resulting in only a few measurements (once per day, etc…). Such analyses limit spatial resolution and do not allow detecting diel shifts (day-night, etc.) in such parameters and small effect sizes of response variables to experimental manipulations may stay undetected or below necessary statistical power. Additionally, manual sampling and probing are costly in terms of necessary man time for sampling and laboratory analyses. Within AQUACOSM and AQUACOSM-plus, several levels of automatization of high frequency measurements of above-mentioned parameters have been already developed or are in progress:

1) LAMP sensor-system suspended in situ within mesocosms

CNRS-MARBEC LAMP (Lite aquatic Automated Mesocosm Platform), permits monitoring physical, chemical and biological parameters at high temporal resolution The LAMP Sensor System can handle a large number of digital probes (e.g. nutrient probes, light sensors, laser diffraction sensor, etc.), offering modular opportunities. This allows flexibility and permits to adapt the system to mesocosms with different diameter (Final Report on LAMP Sensor system in AQUACOSM Deliverable D8.4)

2) Low cost sensor developments

The LAMP system is based on state-of-the-art commercial sensor solutions with very high sensitivity and resolution. The costs of such systems are therefore accordingly in a range that will often not allow a large number of systems operating in experiments with high replication, or to be used in low budget situations. Hence, optimizing costs by establishing cost effective sensor systems from robotics etc. can be a solution for such applications. Potential lower sensitivity and resolution of sensors at low concentrations of parameters can be partly compensated by high measurement frequency, allowing detecting also subtle shifts in parameters after experimental manipulations. Such cost-effective LAMP-sensor systems are currently under development within WP7 of AQUACOSM-plus.

3) Aquabox for automatized analyses of water streams collected within mesocosms

The AquaBox is a compact and modular flow-through system capable of high-precision monitoring of mesocosms. AquaBox combines peristaltic and multichannel valves, flow cells and sequential flow technology, performing autonomous measurements successively from several mesocosms installed in a joint rig. By employing flow-through sensors/analysers and automation software for system control the system can perform multiple measurements on each water sample, allowing for high precision and reproducibility, as well as semi-continuous measurement frequencies with a set of sensors for key properties most prone to temporal variation in planktonic systems during mesocosm experiments (temperature, oxygen, in vivo phytoplankton pigment fluorescence at several wavelengths). Extensions

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include inorganic nutrient analyses (miniaturized wet chemistry), Fast Repetition Rate Fluorometry (FRRF) and Pulse Amplification Modulated (PAM) fluorometry as primary productivity proxies, in-water carbonate system chemistry (pCO_2, pH), dissolved organic matter (CDOM and FDOM), interphase for gas exchange measurements across water surface, flow cytometry, and image analysis of individual cells of phytoplankton and zooplankton species (FlowCAM, CytoSense, FlowCytobot). (Final report on AquaBox in AQUACOSM Deliverable 8.3)

Despite their very different structure and organization all above described systems are capable of performing high frequency measurements of important environmental parameters. Whereas suspended systems are less cost- and logistic intensive, high-tech solutions such as the AquaBox will need higher financial and logistic support. All of these systems have in common that they will allow much deeper insights into environmental dynamics following planned experimental manipulations than "traditional" mesocosm systems can do.

Grand Challenges are mostly related to changes in the abiotic environment (Salinization, acidification, eutrophication) and how communities respond to it. However, responses and physiological and behavioural dynamics of plankton organisms following such changes may happen much faster than often can be captured by traditional measurement methods. A much higher frequency of monitoring both, abiotic and biotic responses to directed experimental manipulations can give better causal insights into Grand Challenges. Automatization involving high frequency measurements is therefore one important step to increase related causal understanding and thereby prediction capabilities.

2.3 Joint mesocosm experimentation:

Grand Challenges are environmental changes influencing important ecosystem services with potential drastic consequences for human populations and are therefore of societal concern. Experimentally analysing Grand Challenges can therefore have direct importance for decisions about reducing stressors related to Grand Challenges and developing mitigation concepts. It is therefore important to have a good overview about robust general but also regional-specific responses of aquatic systems to environmental shifts related to Grand Challenges.

In most cases mesocosm experiments have been performed at a single site at a single time point. That means that most previous experiments have neither been performed in different times at the same site (see however Nejstgaard et al 2003, Larsen et al 2015), or at several sites in a planned and concerted way (see however Landkildehus et al 2014). The outcome of an experimental manipulation performed with a given local community depends on the available species pool, which in turn is affected by various local and historic factors (connectivity of habitats, history of pollution). The results of experimental manipulations thus are influenced by the local environment and the local biological community, where the experiment is performed. Not surprising, the same experimental manipulations done at different sites can even deliver opposing results (also, the same experiment performed at one site at different seasons, see Thingstad & Cuevas 2010 for examples and model considerations). Joint mesocosm experiments will yield more reliable and exhaustive results on how ecological communities may respond to a given pressure.

Strategies to perform joint mesocosm experiments are mostly based on large mesocosm set-ups replicated at different sites. Such a strategy allows to perform a large number of treatments timely at different sites, however it needs careful planning and available options at these sites to perform the experiments. A further addition to such a strategy is a "mesocosm of opportunity" design, a reasonable number of defined mesocosm treatments is added to already planned mesocosm experiments thereby allowing a large number of potential sites joining in spite of already existing other experimental commitments.

Within the project AQUACOSM a pilot study of such a combined experimental strategy has been performed. At various European sites, from polar to Mediterranean region including freshwater, brackish and marine waters harmonized experiments investigate the effects of increasing runoff of terrestrial material (DOC) into aquatic systems. First results point towards clear site-specific patterns following the same experimental manipulation, however effect sizes of responses to the manipulations were surprisingly comparable.

AQUACOSM-plus continues and strengthens such types of approaches by planning further joint experimentation, for example by a strategic cooperation with the SITEs consortium (Sweden) in experimentally tackling defined Grand Challenges (WP8) and concerted TA (WP9) activities.

2.4 Upscaling complexity:

A mesocosm separates an enclosed water column from part of the chemical, physical and biological environment, thereby reducing environmental complexity. The reduced complexity can on the one hand be an important aspect of mesocosm experimental strategies to follow a directed experimental manipulation, for example to test basic ecological concepts or lower food web dynamics. However, on the other hand reduced complexity may at the same time hinder up-scaling to natural environments and it depends on the investigated research question to determine the optimal compromises between these two aspects. However, dealing with Grand Challenges needs up-scaling to natural environments and mesocosm experiments with increased complexity have to be part of the experimental possibilities. Important aspects of complexity can for example include the vertical zonation of a water column into epi- and hypolimnion with very different physical and chemical structure (euphotic, aphotic; mixed, unmixed; oxic-anoxic) and therefore very different important biological process dynamics (photosynthesis, respiration, recycling, nutrient depletion etc.) These are very important processes for up-scaling and deep enclosure systems allowing vertical water column zonation can be an important part of such attempts. A further extension of such approaches is to couple pelagic mesocosm environments with benthic compartments to enable studies of important biological and chemical dynamics between these environments. Complexity can also increase with the enclosed volume within mesocosms as higher trophic levels can be part of investigations. Organisms at higher trophic levels, such as fish, usually require large volumes and are therefore often excluded from smaller mesocosm systems. Finally, upscaling must also consider time scales beyond the duration of experimental manipulation. In a mesocosm experiment, the response of an enclosed community is studied for a given time (usually weeks). This limits responses mostly to demographic changes, while long-term changes such as adaptation, evolution, and changes of the species pool from immigration and extinction, are not considered. Aforementioned experiments at multiple sites are one possibility to explore ecosystem responses under multiple historic conditions. Comparison of experimental data with long-term analysis of ecosystems (LTER, etc.) represents another avenue. The envisioned RI-RI collaboration in AQUACOSM-plus with other networks such as LTER, JERICO and others offers mutual benefits in terms of analysing long-term trajectories in combination with mechanistic understanding under experimental scrutiny. Moreover, several sites performing long-term experiments participate in AQUACOSM-plus (Long term mesocosm research at AU, Denmark; Iberian Pond Network, and its extension in Hungary, WP8).

In the AQUACOSM and AQUACOSM-plus projects both approaches to increase complexity in mesocosm research, mentioned above, have been successfully tested, for example by comparison of benthic-pelagic and only pelagic mesocosm systems exposed to identical experimental manipulations during the JOMEX joint mesocosm experiments in WP9 of AQUACOSM. Lightweight mobile systems build in WP7 of AQUACOSM-plus (LMU) will specifically include options for allowing pelagic-benthic coupling. Additionally, the available consortium mesocosm infrastructure of the AQUACOSM-plus consortium include very large systems (IGB LakeLab, GEOMAR KOSMOS, CNRS CEREEP PLANAQUA) that allow including nearly full food web complexity for experimental analyses.

2.5 Summary/outlook

All of the above-mentioned aspects of experimental designs are explicitly addressed and further developed by various activities in AQUACOSM-plus, including the involvement of RI-RI collaboration, which connects AQUACOSM-plus to other excellent networks. Hence, the consortium is well positioned to bring mesocosm activities within the necessary scientific approaches to work on today's and future Grand Challenges which European and global aquatic ecosystems have to meet.

3. Dissemination Activities Related to this Deliverable

No specific Dissemination activities are planned in regards to this deliverable.

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