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1. Executive summary

Task 1 comprises of workshops on best practices and optimization of mesocosm approaches. The first of these workshops was entitled: Optimizing mesocosm design and operational procedures and took place between the 5th and the 7th of December in Évora, Portugal.

The overall aim of the workshop was to produce recommendations for existing mesocosm approaches, help develop proposed guidelines on mesocosm experimentation and prepare a roadmap towards future work that includes experimentation with mesocosms. The workshop also contributed to task 2.1: Review of the present conceptual state, capabilities and limitations of aquatic mesocosm facilities and specifically MS10 (Review Workshop).

In the DoW specific themes to be addressed are listed as:

1) Identifying the strengths and weaknesses of existing facilities and approaches spanning the full range from river, lake, brackish and marine environments, concerning indoor and outdoor, pelagic, littoral and benthic facilities.

2) Discussing and agreeing on best practices in mesocosm applications, including experimental design, replication, randomization, core parameters to be measured, statistical data treatment and analysis, data reporting and archiving.

As mentioned above, the present workshop combined Task 3.1 with part of Task 2.1. The outcomes of task 2.1 are to be presented in a dedicated report in M17 and the plan was to consolidate the actions so far in a workshop in M12 (milestone 10 “Review of present conceptual state, capabilities and limitations of aquatic mesocosm facilities”). Since the participants who would be present in that workshop coincided with the workshop of task 3.1 the two were merged in order to progress with reviewing the present conceptual state capabilities and limitations of aquatic mesocosm facilities. The plan was to also build on this workshop to contribute towards task 3.4: Perspectives beyond the project, especially regarding the planned “Best practices manual”.

During the workshop it emerged that, in terms of point 1, the AQUACOSM community is at a quite mature stage of recognising the advantages as well as the caveats associated with mesocosm experimentation. Mesocosms provide with the opportunity to control experimental conditions on a scale large enough to typically include three trophic levels or more. Fluxes and processes can be measured, the effects of perturbations on the system can be tested and comparisons can be made with the natural ecosystem. Mesocosms are at the same time often isolated, the effect of water enclosure can compromise results and the divergence from the natural conditions with time, often hinders meaningful extrapolations. The discussion also focused on future perspectives and improvements, such as the potential to use semi-permeable bags and of conducting distributed experiments on multiple scales. Novel systems are also being developed by the community, as for example of the AQUACOSM prototype in WP7.

As for the second point, the need to refresh and integrate approaches from different environments was apparent. An important component of the integration is to merge the insights from marine and freshwater systems, which has started already in the framework of AQUACOSM. There is a lot of ground to cover it terms of describing experimental design data treatment, analysis etc. The aim is to collate existing information from the different approaches in order for new users to decide what fits their needs best and what potential modifications are necessary. Part of this work is directly connected to the work carried out in WP4, specifically the development of Standard Operation Protocols (SOP’s). Another part is carried out within WP3 as part of tasks 3.3 and 3.4. It was stressed that it’s unnecessary to repeat what has been done already; the purpose is to organise and update existing resources and contribute where gaps are identified.

Further, there was consensus that the term “best practises” or “standards” should be reconsidered, as the terms “recommendations” or “guidelines” were deemed more appropriate for the work undertaken in AQUACOSM. The workshop addressed the specific themes with further detailed discussion and planning progressed on how to

incorporate the issues discussed into recommendation and documents that can be utilised by the wider community.

N.B: Following the 1st Amendment to the Grant Agreement, this Deliverable submission date was due Month 11 (NOV 2017). The AQUACOSM Coordinator wrote an email to Agnes Robin 20/06/2017 to request the postponement of D3.2 from Nov 2017 to Dec 2017 as the planed workshop would collide with a major Freshwater event (http://gleon.org/meetings/gleon19/main) where many central actors in AQUACOSM have leadership obligations. Postponement was accepted by the Project Officer 21 June 2017 by email.

2. Workshop details

2.1 Workshop organisation

The workshop was organised by partner 18 (CIBIO-UE) in collaboration with partners 1 (FVB-IGB), 2 (UNI) and 3 (UiB). During the planning period an organising committee was also appointed in order to provide specific input on potential topics to be covered. Partners 11 (UMU), 13 (LMU), 14 (ENS), 15 (AU), 17 (UH), 19 (IMPERIAL) and 20 (NIVA) participated in the organising committee. Communication through email and skype where necessary were used for organisational details, such as invited speakers etc.

The workshop programme was as follows:

5th December
09:00 – Welcome session and logistics
09:15 – Presentation of goals for the workshop
Guest Talks: Strengths and weaknesses of existing mesocosm facilities spanning the full range from river, lake, brackish and marine environments, concerning indoor and outdoor, pelagic, littoral and benthic facilities for studies of:
9:30 – Ecosystem functioning (Robert Ptacnik, WCL)
9:50 – Pollution (Ketil Hylland, University of Oslo)
10:10 – Climate change (Ulf Riebesell, GEOMAR Helmholtz Centre for Ocean Research Kiel)
10:30 – Coffee break
Guest talks on best practices in mesocosm applications:
11:00 – Experimental design, replication, and randomization (Antony Underwood, University of Sydney)
11:20 – Core parameters to be measured (Martin Wahl, GEOMAR Helmholtz Centre for Ocean Research Kiel)
11:40 – Data treatment and analysis (Pedro Peres-Neto, Concordia University Montreal)
12:00 – Modelling practices (Selina Våge, University of Bergen)
12:20 – Data reporting and archiving (Rob Thomas, Marine Institute of Ireland)
12:40 – Lunch
14:00 – General comments, preparation for breakout groups (Miguel Araújo, UÉ)
14:30 – Parallel breakout group sessions
17:30 – End of sessions
19:30 – Social dinner

6th December
9:00 – Parallel break out group sessions
10:30 – Coffee break
11:00 – Parallel break out group sessions
12:30 – Lunch
14:00 – General discussion and synthesis for groups 1
15:30 – Coffee break
16:00 – General discussion and synthesis for groups 2
17:30 – Conclusions from break out groups
19:30 – Social Dinner

7th December
09:00 – Welcome & Background:

- Tasks, milestones and deliveries related to this action in the project
- History of the book idea and editors
- Possible publishers and initial ideas about alternative publication forms

- Suggested initial Book layout

09:30 – Contributors presenting suggestions
10:30 – Coffee break
10:45 – Contributors presenting suggestions (continued)
12:30 – Lunch
14:00 – Contributors presenting suggestions (continued)
15:00 – Preparation for breakout groups
15:15 – Coffee and breakout in discussion groups
16:15 – Summary of discussion groups, general discussion and way forward
17:00 – End
19:30 – Dinner

2.2 Summary report

2.2.1 Day 1- December 5th Summary

The diverse purposes for which mesocosm experiments can be used to answer pertinent ecological questions were highlighted during the first set of talks on December 5th. The degree of control over the development of ecosystem dynamics is very useful, as there are many components of food webs that we lack detailed data on.

2.2.1.1 Talks on major thematic areas mesocosms are utilised for

The first three talks focused on case studies and examples of thematic areas where the use of mesocosms has consisted of a powerful tool moving research forward. Ecosystem functioning, pollution and climate change were the three topics covered.

For ecosystem functioning, examples were discussed where mesocosm experiments are used to answer questions about patchiness, ecosystem connectivity and dispersal. The evolution of communities and the connections between biodiversity and ecosystem function can be studied using the mesocosm approach. Considerations using this method for ecosystem functioning experiments should include potential loss of biodiversity, especially over time, in enclosed systems and selection of species based on the enclosure effect. Gradients were recommended for the better study of biodiversity and ecosystem function relations.

Although the degree of control over a community is unique using the mesocosm approach constraints include the duration of the experiment and limitations in the levels of environmental and biological complexity.

During the discussion it was noted that very often, when proxies are used instead of certain parameters e.g. using biomass accumulation as a proxy for productivity, which could result in misinterpretation of the obtained results. Selection through enclosure was also debated, as one could argue that the enclosure itself makes the conditions ideal for some species to prevail and is not related to fitness environmental functions.

In order to deal with confounding factors the question of what we would do differently given the resources was raised. This became an underlying theme to all subsequent discussions and a major issue to consider when thinking of future research.

The next talk was on the utility of mesocosm approaches to answer questions about the abiotic behaviour of toxins and particles. In this type of experimentation, stressor interactions can be monitored and the effects of pollution on biological processes can be quantified. Realistic pollution scenarios can be tested during experiments with substances one would not be able use for tests in the aquatic environment. Pollutants released in aquatic environments call for studying stressor interactions, flow of toxicants in the food chain and effects on processes, all of these can be very adequately studied in enclosed systems, up to certain trophic level.

Discussion revolved around confounding factors including water aging and wall growth as well as what we should be measuring to answer questions about toxicity and ecosystem functionality. This question is pertinent to studies on pollution since the effects of pollution are not always visible in organisms and many effects take a longer time to "translate" into measurable effects.

The third thematic area was climate change. Different drivers and stressors can be tested, the limitation here is that individual drivers are well studied but not necessarily on a community level, we lack mechanistic understanding of the effects of climate change on a community level. It was underlined that many of the climate change experiments are focused more on scenario testing rather than understanding community responses. An issue to consider when working with multiple stressors and drivers is disentangling the type of effects one can expect, whether they are synergistic, antagonistic or cumulative. This can become a major problem; it is largely ignored in many studies even though longer term trends are emerging. For example, meta-analysis of interactions in marine systems shows climate change effects are mostly synergistic whereas in freshwater systems they tend to be mostly antagonistic.

It was suggested that the response curve of each organism of interest in the experiment should be established before any multi-stressor effects can be inferred. Lack of knowledge of the response curves can lead to the production of meaningless data. Also perturbations should be ecologically meaningful and vary dynamically with the environment and season they are tested in.

During the discussion a point that emerged was the relevance and applicability of climate change experiments and their contribution to mitigating and / or solving emerging issues. Solution oriented research and steps towards ecosystem restoration were subjects that came up, highlighting the responsibility of science and research to contribute more in those areas.

2.2.1.2 Talks on design and methodological considerations and approaches

The second part of the day consisted of three more methodological talks on the subjects of experimental design, core parameters to measure and data treatment and analysis. Regarding experimental design, it was described there is much room for mistakes which can lead to inaccurate result interpretation. The volume of information produced in the last decade alone makes it difficult to filter out which experimental results are meaningful, reproducible and appropriately replicated and treated.

Hypothesis building is as important as the experiment itself and design is crucial not only to the reliability of the outcome produced but also to a successful experiments since wrong design can mean we are answering the wrong questions. In every experiment it is essential to properly consider and address replication, independence, appropriate controls and the appropriate sample size, among other considerations.

Updated approaches to experimental design and statistical treatment were discussed at length but the need was also expressed to provide advice as to what steps to take when there is a deviation from initial planning. Efforts should be made to also advise on what to avoid and how to mitigate potential deviations while an experiment is in progress.

The core parameters talk outlined how knowledge of the system the experiment takes place is very important as well as the time of experimentation, since there should be extra care given to extrapolating the results to more generalised patterns. The recommendation was that an idealised experiment is initially considered and as we modify to available time and resources we document what we are losing in terms of experiment robustness. There is a gap between what is desirable and what is feasible, but we should now how much we can cut back without compromising the findings.

The experimental design should also determine which parameters should be measured along with the studied community. In terms of measured parameters the recommendation was to consider e.g. covariance among treatments, which could potentially decrease as stressor intensity increases. Another recommendation was to consider add- on experiments to answer questions that could make the data interpretation more meaningful. The

collapsed factorial experimental design, as described in Boyd et al (2015) was suggested as a potential design that would fit experiments using mesocosms. Logging some parameters continuously was also mentioned as a good practice when feasible. The parameters that need to be measured of course depend on the questions posed before the experiment and the community studied.

The talk on statistical analysis focused on considerations when working with the produced data. It follows that appropriate experimental design should give insight on what should be measured and if correct replication and randomization are followed, the statistical analysis should give us the desired answers. Autocorrelation between samples should be addressed and although different approaches can be utilised depending on the experimental design, it is important to think which analysis is appropriate for the dataset produced. For mesocosm experiments, the fourth corner approach (Dray et al, 2014) was suggested as an approach to use when trying to assess trait responses to environmental gradients.

The next talk, touched upon the use of conceptual models in conjunction with mesocosm experimentation Conceptual models can be useful in determining the hypothesis to be tested, in taking ecological theory further and determining what gaps in ecosystem functioning and trophic interactions we can cover through an experiment. The minimum model (Thingstad et al, 2007) was presented as an example where the least combination of trophic connections is used to explain ecosystem functioning. The level of detail is flexible, depending on the question. Whether we want to simplify when predicting ecosystem responses to bottom up and top down manipulations as well as when there is need to focus on complexity such as host-virus interactions or competition and defence trade-offs, a conceptual model assists with theory formulation and result interpretation.

An additional consideration presented was the concept of model performance and model inter-comparison. As performance is usually evaluated based on the dataset used to build the respective model, an independent set of standards has been set up to evaluate model robustness.

Finally, data management practices were presented, as the reporting collecting and disseminating information is crucial to this consortium. Keeping good data practices was underlined as being as crucial as collecting the data itself. Within reasonable time the data should be made publicly available while chain of evidence should also be practiced in order to ensure that data collected is timeless and can be reused and revisited as necessary.

The FAIR principle was recommended as the modus operandi for AQUACOSM, an approach adopted by data centres worldwide. FAIR refers to data being Findable, Accessible, Interoperable and Reusable, as the availability of datasets is now a pre-requisite in many journals it was recommended the principle is adopted before any of the data collections starts.

The invited talks had the overall aim to present the state of the art in topics of interest and to provoke thought on how to move forward with constructive recommendations and future challenges. A discussion in plenum followed the talks, reflecting on and reiterating what had already been discussed during the day.

Some general questions that emerged from the discussion that the community should endeavour to answer:

What types of hypotheses can we test using a mesocosm approach?

What are the main limitations of this experimental approach?

What steps can we take to minimise the possibility that our results cannot be distinguished by noise are auto correlated etc?

Which parts of the terminology and jargon we use are poorly defined?

But also:

What would be our desired course of action in the event the funding was available?

What can we do to contribute to solutions in addition to pointing out what the problem is?

Moving forward it was decided to split into two groups for the next day. In order to effectively address obligations to tasks 2.1 and 3.4 it was decided that the two groups should be discussing in terms of the manual the

consortium envisions to produce, comprising of recommendations and updates on practices where mesocosm experiments are required. The theme of group one was “experimental planning” and the theme of group two was “practical considerations”.

The format of the guidelines was discussed an online downloadable format is desirable and the suggestion to create a wiki was also very favourable. It would help if a dialogue can be initiated with the wider community that twill then endorse the standards or guidelines produced and endorse them.

Finally, as part of task 7.1 the design of a prototype AQUACOSM should be discussed during the present workshop. Partner 11, UMU presented the prototype constructed and is being tested presently and participants discussed with the involved partners further considerations and course of action for the coming months.

2.2.2 Days 2 & 3- December 6th and 7th summary

As discussed during day one, the next two days were dedicated to discussing on specific aspects of the recommendations that will be made publicly available, on experimental design and data analysis. Our working title was: The use of mesocosm in aquatic research.

2.2.2.1 Group one- Experimental planning summary

As part of the experimental planning five key points were discussed and deemed crucial to consider when undertaking experimental work using mesocosms.

Point 1- Knowing the ecosystem where the experiment is taking place, should include:

- Keystone species
- Seasonal patterns
- Observational data
- Times series/ Historical data if available
- Modelling data
- Conceptual model for hypothesis building

Point 2- Setting up the questions should include:

- Setting up the alternative and null hypothesis
- What is the most appropriate way to test the hypothesis
- Statistical framework
- Experimental units
- Required data if models will be tested
- Minimizing artefacts

Point 3- Statistical design, replication, randomization, should include:

- Within and between variance
- Estimate variation
- Minimize residual variation
- Identifying and handling outliers

Point 4- Data handling, should include

- Quality assurance
- Quality control

Point 5- Data legacy, should include:

- reporting and archiving
- FAIR principles
- complete and updateable datasets
- acknowledgements

2.2.2.2 Group 2- Practical considerations

Practical considerations were centred on the choice of the correct mesocosm system, depending on the question that needs to be answered and on the construction and operation of a mesocosm experiment.

Point 1- Choice of system, should include:

- Pelagic
- Sediment
- Hard-bottom
- Combinations of the above
- Duration and timing of experiment

Point 2- Ex situ- Land based system, should include

- Indoor and outdoor systems
- Groundwater
- Ices
- Running water & Artificial streams
- Tanks, tubes and ponds
- Combinations

Point 3- In situ systems, should include:

- Running water
- Pelagic systems
- Bentho- pelagic systems
- Benthic and littoral combinations

Point 4- Construction and operation, should include

- Shape, dimensions and materials
- Filling and set up
- Physical manipulations
- Chemical manipulations
- Biological manipulations
- Sampling and measurements
- Invasive sampling

Point 5- Areas of special interest, should include

- Acidification
- Eutrophication
- Invasive species
- Trophic cascades
- Stoichiometry

For the last point it was discussed that a series of case studies or success stories should be included as well as examples of failures and lessons learned.

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2.3 Further considerations

The points outlined in section 2.2.2 were discussed thoroughly during the two last days of the workshop. Contributors agreed that by the end of February they will elaborate more on the areas of their expertise. During the next AQUACOSM general assembly an updated timeline will be discussed with all partners present.

This workshop was met enthusiastically from all the partners and the participation was larger than expected, this is very positive in terms of initiative and partner engagement but also means there are a lot of opinions voiced and comments to consider. Despite the large breadth of expertise present, there was consensus that future steps should at all costs, avoid “reinventing the wheel” in terms of the recommendations the consortium wishes to produce.

The workshop successfully addressed the intended aims, and lay the initial plans on how to best gather the collective knowledge to effectively develop scientifically authoritative guidelines for future best practice aquatic mesocosm based science in EU and world-wide.. This process will be continued both during the best practice WS and specifically through the planned Book-writing process, aimed to result in an modern open web-based and peer-reviewed and fully citable document, to be further elaborated in the next WS in connection with the GA-meeting in March 2018.

3. Dissemination activities related with the Deliverable

Detailed information about the workshop, including the goals and the program, was disseminated thorough the project's web portal: https://www.aquacosm.eu/events/workshop-1-optimising-mesocosm-design-and-operational-procedures-wp3/

Updates of progress during the workshop were also twitted using the official @aquacosm twitter account (Tweet 13 December 2017).

4. Appendix

University of Évora W 2 & 3 AQUACOSM Workshop

Folha de Presenças/Attendance

Nome/Name	05-12-2017	06-12-2017	07-12-2017
Anastasia Tsiola			
Antony Underwood			
Behzad MOSTAJIR			
Corantin Soton			
Gérard Lacroix			
Hartvig C. Christie			
Iordanis Magiopoulos			
Jens C. Nejstgaard			
Jeremy Piggot			
Johan Wikner			
Jose Gonzales Fernandez			
Joseph Huddart			
Jukka Seppala			
Ketil Hylland			
Lisette de Senerpont Domis			
Maria Stockenreiter			
Marko Reinikainen			
Martin Wahl			
Meryem Beklioğlu			
Miguel Araújo			
Miguel Matias			
Pedro Peres Neto			
Rob Thomas			
Robert Ptacnik			
Sarah Fiorini			
Selina Vage			
Stella Berger			
Tatiana Tsagaraki			
Thomas Davidson			
Timo Tamminen			
Ulf Riebesell			

Jorun Egge

UNIVERSIDADE DE ÉVORA

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