NKS-373
ISBN 978-87-7893-458-1

NKS-B NORCO Mid-term Report
Nordic freshwater ecosystem microcosms study

Hevrøy, T[1]
Bradshaw, C[2]

[1] Norwegian Radiation Protection Authority
[2] Stockholm University

August 2016

Abstract

This mid-term report is an overview of the progress of the NKS-NORCO research project sponsored by the NKS-B program. This project set out to test and create realistic microcosms of ecological systems that can be used as a reference point for future microcosm studies in the field of radioecology, specifically using Nordic freshwater ecosystems. To achieve this we have carried out multiple tests of different potential species, to determine which species remain stable in cultures and when combined in multispecies microcosms. Furthermore, we have tested the impact of different nutrient levels, abiotic parameters and sediments on biotic parameters. This report summarizes our initial results from testing a variety of species and measuring the structural and functional parameters in microcosms not exposed to a stress. These results will aid us in better understanding the natural fluctuations within our microcosms, which will provide a basis for any effects we may witness when microcosms are exposed to a stress. We plan to expose microcosms to radiation at two different facilities (in FIGARO in Norway and Stockholm University in Sweden) in order to compare and calibrate the facilities, exchange knowledge and competences, and standardise procedures between the organisations involved. Due to physical restrictions of the test facilities, the microcosms exposed in FIGARO will be 4L and those exposed at the Stockholm University system, 1 L. Microcosms will be exposed in October 2016 to four different dose rates, ranging from 39mGy/hr to 0.67mGy/hr. The aquatic microcosms will contain a selection of aquatic plants, phytoplankton, zooplankton and aquatic snails. Structural and functional effects will be measured, some at different time points throughout the experiment, others only at the end since destructive sampling will be required. The involvement of two radiation protection authorities (NRPA and STUK) in designing the experiment will also ensure that the approach and results are of use to regulators and assessors.

Key words; radioecology, radiation, microcosm, freshwater, ecosystem

NKS-373
ISBN 978-87-7893-458-1
Electronic report, August 2016
NKS Secretariat
P.O. Box 49
DK - 4000 Roskilde, Denmark
Phone +45 4677 4041
www.nks.org
e-mail nks@nks.org

1. Overview

The specific aims of this project are to carry out pilot studies using external gamma radiation exposure of aquatic microcosms to:

a) evaluate the practical usefulness of microcosms in a radioecology context
b) obtain new data on ecosystem level effects for freshwater Nordic ecosystems
c) standardise methods and protocols to be used in multi-partner studies in the future.

In the NKS-NORCO project so far, the first point has been our focus point. Specifically, we have been researching species combinations, setting up stable cultures, identifying significant parameters and assessing natural variability in microcosms without a stress added.

Partners:

1. Norwegian Radiation Protection Authority (NRPA)
2. Stockholm University (SU)
3. Norwegian University of Life Sciences (NMBU)
4. University of Eastern Finland (UEF)
5. Radiation and Nuclear Safety Authority (STUK)

Outline of report

1.1 Kick-off meeting and following discussions

Firstly, members of the NKS-NORCO project team met on February 10th, 2016 in Oslo, Norway for a "kick-off" meeting. The main items on our agenda for this meeting was;

- Introductions – round table summary of each team members background and contribution to the project
- Re-acquaintance of the project aims and outlining of hypothesis
- Radiation facilities (FIGARO and SU) – availability and restrictions
- Microcosms – initial pilot tests, species combinations, abiotic factors
- Endpoint measurements: direct and indirect.

1.2 Test facilities

The FIGARO facility was officially opened in December 2012. The facility has a 12 Ci Co-60 source which provides a continuous dose rate field from 3 Gy/hr (at source) down to 400 µGy/hr (Fig. 1). The test hall spans 20 meters in length, with adjoining control room and laboratory facilities. The climate control specifications for the experimental hall are:

Temperature: 4 - 37 °C (+/- 1 °C)
Light : ca. 50 - 300 lux with automatic dimmer (10 min)
Humidity: 45 - 65%
Ventilation: 300 m^3/h

The field of width of the beam is a significant parameter; at 2 meters from the beam the field of width is only 40cm expanding to almost 3m at 15 meters from source (Fig.1). Taking into account optimum number of exposures, shielding effects and rate of loss of gamma dose through water (47% reduction through 10cm water), we have had to significantly reduce the size of the microcosms originally proposed in this pilot study. The maximum cosm depth (relative to the beam) recommended by experts (pers.comm Hans Bjerke and Per Otto Hetland, NRPA) is 10cm, in order to avoid excessive attenuation of the gamma dose through the media. To achieve a high enough dose as to expect biological impacts (FIGARO individual species tests have not shown any effects below 10mGy/h) and eliminate any shielding effects the microcosms cannot be wider or higher than 20cm. We will therefore now be exposing 4L aquaria at four exposures (Fig.1), instead of 25L microcosms we intended on in our proposal. Although this is a significant reduction in size, the species complexity we can achieve in 4L aquaria does not differ from the species complexity we had outlined in our proposal for 25L aquaria.

The facility is in high demand and the will not be available to us until autumn 2016. Due to this, our initial timeline has been altered and we will expose the microcosms to radiation for 3 weeks (with a one-week stabilization period) for the duration of October. Nevertheless, this delay has provided us with more time to run pilot experiments on microcosms before exposure to radiation, allowing us to explore in greater detail the natural fluctuation in abiotic and biotic environment within microcosms, the parameters we need to control and explore all the potential endpoints.

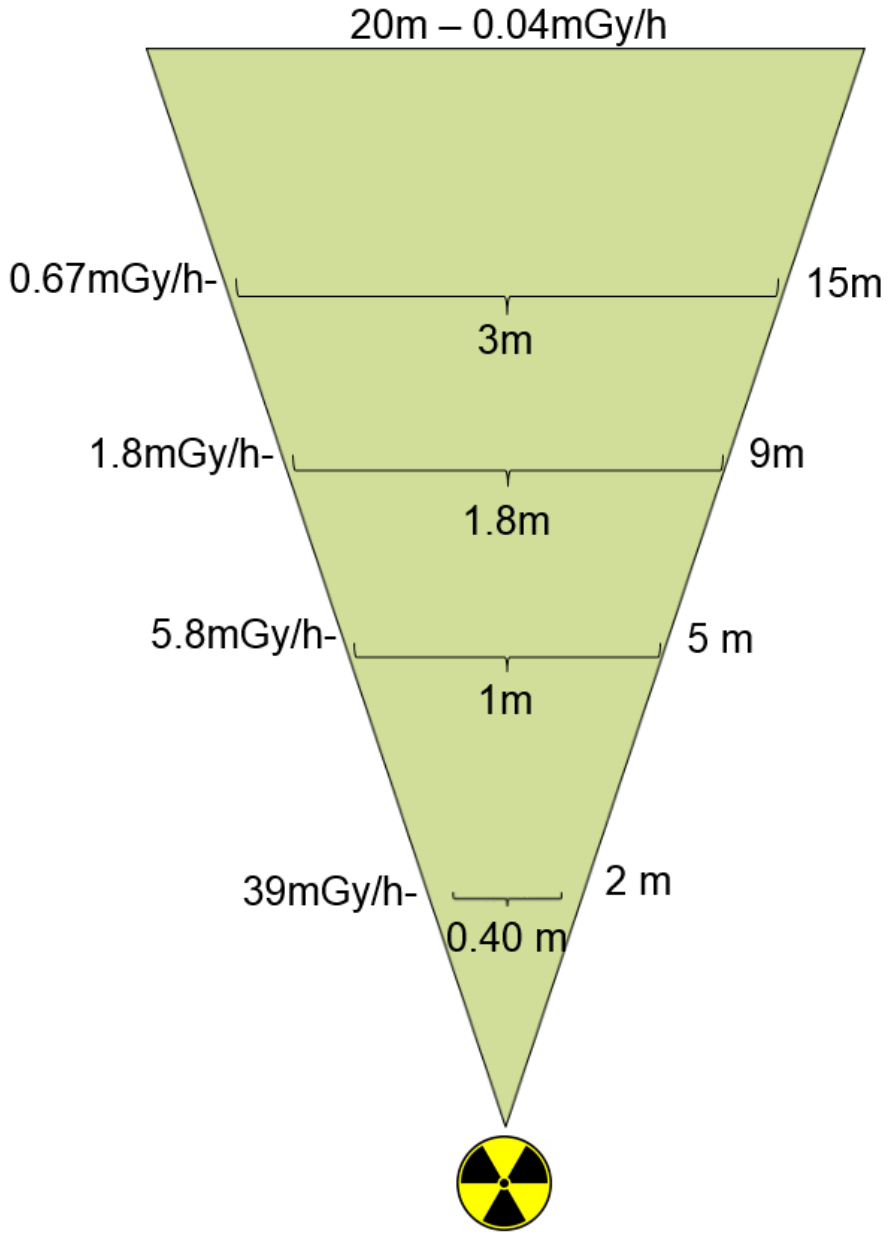

Figure 1. FIGARO radiation facility. 20-meter room with radiation beam to scale illustrating field of radiation beam width and dose rates at different distances from source.

The Stockholm University Radiation cabinet is 125cm x 56cm x 36.5cm and has a Cs-137 source at the bottom of the cabinet giving a dose rate ranging from 72mGy/hr to 12.5mGy/hr (Fig. 2). Based on the dimensions of the cabinet, exposure levels and keeping in mind shielding, we have calculated the maximum size for microcosms in this pilot to be 17cm x 9cm x 9cm (HxWxD) giving a capacity of approximately 1 litre. Because the radiation source is at the bottom of the cabinet, we have to take into account the impact of sediment on the reduction of dose rate through the microcosm. Unfortunately, in May this year the cabinet at SU has been subject to repairs, which will continue until the end of 2016. Therefore, this system is no longer available to us until next year. The NORCO partners at SU will however continue testing their microcosm pilot tests (without radiation) in parallel to the NRPA.

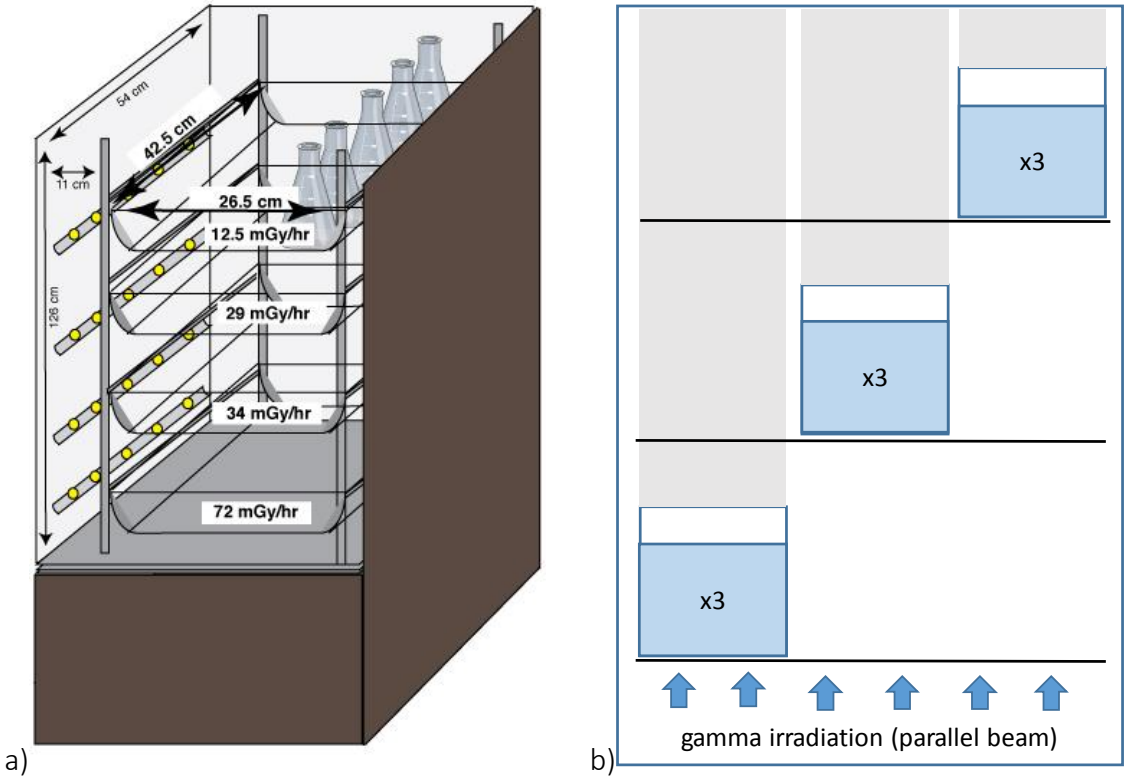

Figure 2. a) Stockholm University Radiation cabinet. Source at the bottom of the cabinet. An identical cabinet without a radiation source is located next to the radiation cabinet, acting as a control. b) Sketch of how the shielding (in grey) and cosms will look at the SU facility.

2. March – April Activity

Our first course of action was to run pilots with different complexity (species composition), in small and larger aquariums with/without additional nutrients and sediment to find the best set-up. These pre-pilot tests were carried out by partners at Stockholm University and the NRPA. Partners at SU had run some pre-trials in January on very simple 2L microcosms that included sediment, aquatic plants and phytoplankton with normal tap water and no added nutrients. (Fig. 3). These initial tests showed stability and highlighted the benefits of aquatic plants instead of using oxygenators.

Figure 3. Pre-testing 2L microcosm trials with sediment, freshwater and aquatic plants at Stockholm University.

We set out to assemble a species composition using species known to occur in Nordic waters and that we know interact in nature (snails, biofilms, aquatic plants, phytoplankton, and freshwater crustaceans. This required contacting various scientists, facilities and suppliers in Scandinavia who housed the species we could use (universities, research institutes etc.).

We tested whether we were able to produces stable cultures of the species listed below at Stockholm University laboratories, whilst NRPA set up a laboratory and equipment for their own tests. The species tested were:

Zooplankton: *Daphnia pulex, Daphnia magna*

Phytoplankton: *Raphidocelis subcapitata, Eustigmatos* sp.*, Chlamydomonas reinhardtii*

Plants: *Myriophyllum hipparoides, Limnophila heterphylla, Lysimachia nummularia, Elodea canadensis, Egeria densa, Hippuris vulgaris* and *Lemna minor.*

Snails: *Lymnaea peregra*, *Gryaulus acronicus, Theodoxus fluvialitis* and *Planorbarius corneus*

It was essential the two facilities coordinated on keeping the conditions for both pilot studies are similar as possible, as well as controlling that all the abiotic factors could be maintained at the same levels in the FIGARO hall. These factors include;

- Lighting (both intensity, type, wavelength spectrum and light:dark cycle)
- Standard sediment
- Freshwater – tap, deionized and/or with and without nutrients.
- Temperature

3. May – July 2016 Activity

NRPA set up their own laboratory based on the initial tests by SU. The same measuring equipment was ordered, similar light and temperature conditions applied and cultures set up. In addition, both labs attempted to obtain species from the same sources, so genetic variability was reduced between laboratories.

The NKS team met again in June (in combination with a visit to the Stockholm lab by Tanya Hevrøy who is running the NRPA lab pilots). We revised results from initial SU tests, potential endpoints were discussed in greater depth and the different roles of NKS partners during the October exposure experiment were planned. Furthermore, the NRPA and SU partners coordinated that their labs are following same procedures and stocks of species and media were transported back to NRPA.

The microcosms were then assembled at Stockholm University (1L cosms) and have been monitored for 4 weeks. The same procedure will be carried out at the NRPA labs (4L cosms) in August (delay due to unstable algal and *daphnia* cultures).

3.1 Species tested

From our original proposal, we set out to create an ecosystem that contained a multitude of direct and indirect pathways by which individual organisms can be affected, including the potential for complex interactions across multiple trophic levels (Fig. 4).

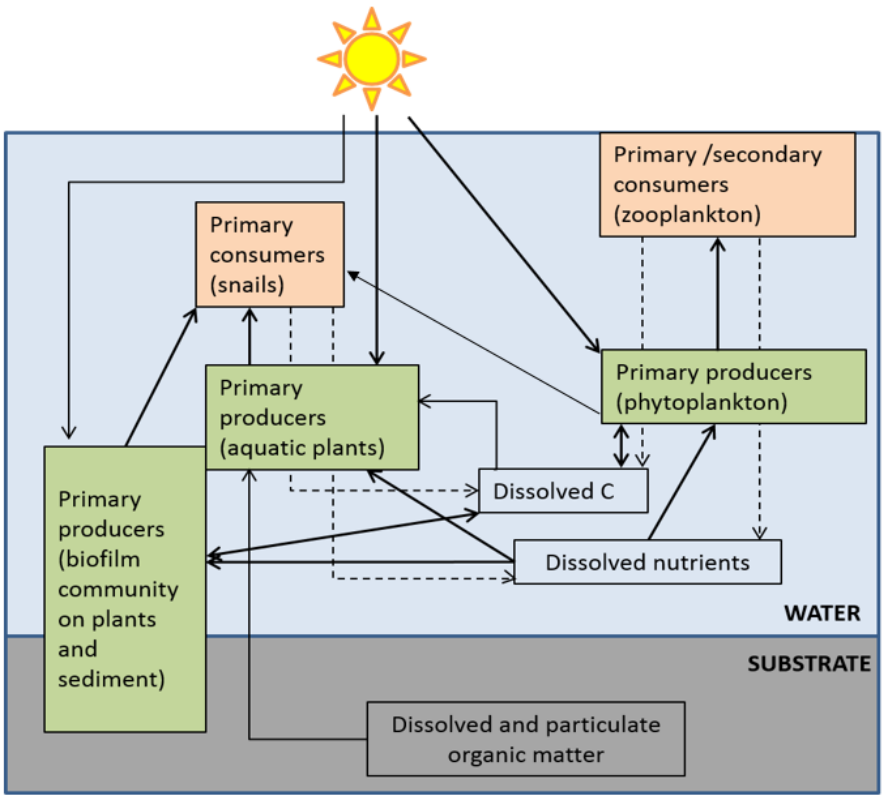

Figure 4. Concept model of the Norco microcosm. Solid lines = trophic transfer and dotted line = excretion/decay.

We have sourced and have stable cultures at both SU and NRPA labs of all the species necessary to fulfil the concept model in Figure 4, and are currently optimising the best combination of these species to create a stable microcosm (Table 1).

Table 1. List of species, species source and the abiotic conditions of microcosms at the NRPA and SU facilities.

Biotic	NRPA	Stockholm University	Indigenous
Lymnea peregra	Aquaculture - Daniel Wæge	SU	Local to Scandinavia
Gyraulus acronicus	Aquaculture - Daniel Wæge		Local to Scandinavia
Theodoxus fluvialitis		Aquashop Stockholm	
Planorbarius corneus		Stockholm Herbarium	
Elodea canadensis	Aquashop Stockholm	Aquashop Stockholm	Weed in Scandinavia
Egeria densa	Aquashop Stockholm	Aquashop Stockholm	Weed in Scandinavia
Lysimachia nummularia	Aquashop Stockholm	Aquashop Stockholm	Native to Europe
Hippuris vulgaris	Tropex Garden Plants		Native to Europe
Lemna minor	Tropex Garden Plants	Stockholm Herbarium	Native to Europe
Chlamydomonas reinhardtii	SU	SU	Native to Europe
Raphidocelis subcapitata	SU	SU	Native to Europe
Daphnia magna			
Daphnia pulex	SU	SU	Native to Europe
Abiotic			
Sediment - fine gravel	Gravel- sterilized (Aquarium shop)	Fine gravel - sterilized (Aquarium stockholm)	
Water	Tap	Tap	
Cosm	Plexiglas - 4L	Plexiglas -1L	
Light - LED	750-850 Lux 16:8 cycle	800-840 Lux 16:8 cycle	
Temperature	18.5-20 degrees	20 degrees	

3.2 SU Microcosms

From previous studies using microcosms indicates that there is no set guideline to using sediment or adding nutrients, as this varies significantly from study to study. The aim of this pre-pilot study was to test whether sediment and/or nutrients were beneficial for the stability of the microcosms. Eight microcosms were set up with the same species composition but different nutrient/sediment combinations (Table 2). The water quality (pH, chlorine, nitrates, O^2, chl a), temperature and the health of the plants and numbers of *D. magna* are being monitored.

Table 2: Sediment and nutrient effects in microcosms. Each of the eight nutrient/sediment combinations contained the following species.

1* *Elodea canadensis*	6 cm shoots	
2* *Egeria densa*	9 cm shoots	No branches and no roots
1**Lysimachia nummularia*	10 cm shoots	
1**Lysimachia nummularia*	15 cm shoots	
4* *Daphnia magna* juveniles (~ 3 days old)		
1* adult *Daphnia magna*		
0.64 mg C *Raphidocelis subcapitata*		
Nutrients/sediment - 8 combinations: Either 1 nutrient pellet or 250 ml MWC medium		
2.5L water in each cosm with/without 1 cm sediment		
2* no sediment no nutrients		
2* sediment no nutrients		
2* sediment with nutrients (1 pellet, 1 MWC)		
2* no sediment with nutrients (1 pellet, 1 MWC)		

Figure 5. Pre-pilot tests at Stockholm University of sediment and nutrients on microcosms with species combination from Table 2.

4. Current status and plans prior to the gamma exposure experiments

The SU pilot microcosms are currently being monitored, as described above. Water nutrient analyses and chemistry will also be measured. The NRPA will set up a similar trial in August based on initial results from Stockholm pilots. The NRPA microcosms will optimize conditions and trial microcosms with slightly altered species combination to optimize the concept

diagram (Fig. 4) and increase ecosystem complexity. This will include different freshwater plants, two phytoplankton species and two snail species. These microcosms will be monitored for the same parameters are SU microcosms have been, including numbers and health of snails and algae concentration. Other endpoints such as *Daphnia* and snail grazing rates and net primary production will also be tested and measured. Furthermore, our partners at NMBU will carry out water chemistry analyses.

Another pilot study will be carried out where the microcosms will be exposed to a known toxic substance (e.g. Cd) in order to test whether the ecosystems have the capacity to respond to stress in a measurable way.

5. Summary

The pilot studies have been very useful in identifying suitable species and appropriate abiotic conditions, and have enabled us to start testing methods for measuring relevant endpoints. This will enable us to have a robust experimental design for the forthcoming experiment at FIGARO in October. The FIGARO experiment will be carried out by team members from NRPA, NMBU, SU and UEF and will include three PhD students (from NMBU, SU and UEF) and potentially one Masters student from UEF.

Title	Mid-term report for Nordic freshwater ecosystem microcosms pilot study.
Author(s)	Tanya H Hevrøy[1] and Clare Bradshaw[2]
Affiliation(s)	[1] Norwegian Radiation Protection Authority (NRPA) [2] Stockholm University
ISBN	978-87-7893-458-1
Date	August 2016
Project	NKS-B/NORCO
No. of pages	11
No. of tables	2
No. of illustrations	5
No. of references	0

Abstract
max. 2000 characters

This mid-term report is an overview of the progress of the NKS-NORCO research project sponsored by the NKS-B program. This project set out to test and create realistic microcosms of ecological systems that can be used as a reference point for future microcosm studies in the field of radioecology, specifically using Nordic freshwater ecosystems. To achieve this we have carried out multiple tests of different potential species, to determine which species remain stable in cultures and when combined in multispecies microcosms. Furthermore, we have tested the impact of different nutrient levels, abiotic parameters and sediments on biotic parameters. This report summarizes our initial results from testing a variety of species and measuring the structural and functional parameters in microcosms not exposed to a stress. These results will aid us in better understanding the natural fluctuations within our microcosms, which will provide a basis for any effects we may witness when microcosms are exposed to radiation.

Key words radioecology, radiation, microcosm, freshwater, ecosystem