Evaluation of fate of nitramines in soil and freshwater

Part 3.3: Biodegradation of nitramines in lake water.

Background and rationale

Nitramines may enter surface waters via various routes, and thus affect the biota in these systems. Moreover, it may contaminate drinking water supply and thus pose a human health problem. There is scarce knowledge of the fate of nitramines in freshwater, however, and the rate of adsorption or degradation. In principle, degradation may occur photochemically (notably by short-wave solar radiation), by other means of chemical reactions or by microbial degradation (bacteria and fungi). Since the absorption spectrum of nitramines is reported to be below wavelengths present under natural, underwater conditions (i.e. 380 – 700 nm), we focussed on microbial degradation in this pilot study. Microbial activity in water is generally dependant on temperature and substrates, where the access to phosphorus (P) and dissolved organic carbon (DOC) are the key parameters. Hence this test was run under a factorial, experimental design at two temperatures and along gradients of P and DOC. Since nitramines also could have potentially harmful effects on biota, we also performed additional pilot studies on key test organisms in aquatic toxicology surveys, the crustacean *Daphnia* and the green algae *Selenastrum*.

Material and Methods

Water was taken from a natural lake (Maridalsvann) and a gradient of dissolved organic carbon (DOC, humus) as well as total phosphorus (TP) were made to control for microbial degradation along these two major gradients of natural waters. The DOC was created by adding standardized, freeze-dried organic matter (0, 10 and 20 mg C l^{-1}) to lake water, and the TP gradient correspondingly by adding inorganic P over a gradient of 0, 25 and 50 µg P l^{-1}. Inorganic nitrogen was also added to "balance" the microbial demands relative to P-additions (Redfield, C:N:P gives 106:16:1) at max P. The experiment was designed as a factorial set-up, i.e. a matrix with all combinations of DOC and TP (totally 9), at temperatures of 5 ^{o}C and 15 ^{o}C in temperature-controlled incubators, see figure 1. The rationale for this set-up is that it will capture the main gradient of water quality and temperature span in lakes.

Table 1. The factorial set-up i.e. a matrix with all combinations of DOC as mg C l^{-1} and TP as µg P l^{-1} for each temperature. The same concentration of nitrogen (350 µg N l^{-1}) was added to each glass beaker.

0 mg C l^{-1} **0 µg P l^{-1}** **350 µg N l^{-1}**	**10 mg C l^{-1}** **0 µg P l^{-1}** **350 µg N l^{-1}**	**20 mg C l^{-1}** **0 µg P l^{-1}** **350 µg N l^{-1}**
0 mg C l^{-1} **25 µg P l^{-1}** **350 µg N l^{-1}**	**10 mg C l^{-1}** **25 µg P l^{-1}** **350 µg N l^{-1}**	**20 mg C l^{-1}** **25 µg P l^{-1}** **350 µg N l^{-1}**
0 mg C l^{-1} **50 µg P l^{-1}** **350 µg N l^{-1}**	**10 mg C l^{-1}** **50 µg P l^{-1}** **350 µg N l^{-1}**	**20 mg C l^{-1}** **50 µg P l^{-1}** **350 µg N l^{-1}**

Two types of nitramines were used in the experiment; DMA-NO_2 and MEA-NO_2. In the first run, DMA-NO_2 was added in 250 ml glass beakers to final concentration of 0.5 mg l^{-1}. From these 18 set-ups, samples for analysis were taken after 0 day, 1 day, 3 days, 1 week, 2 weeks and 4 weeks. Samples (in total 99) were frozen in cryo-vials and shipped for quantitative analysis of DMA-NO_2 by using GC-MS preformed at NERI (The National Environmental Research Institute (NERI), University of Aarhus, Denmark. Subsequently, the experiment carried out again with MEA-NO_2 and samples (in total 99) were frozen in cryo-vials and stored at the University of Oslo. Samples for microbial diversity were frozen for eventually later analysis of microbial community composition and functional genes related to nitramine oxidation.

Results and discussion

The analysis of nitramines gave no straightforward conclusion with regard to degradation. Despite the fact that the applied concentrations were well above the given detection limits, there were analytical problems at the NERI-side. Hence we decided to initially screen only time zero and day 1 to look for short term effects, plus the final date (after 4 weeks) to check for eventually long term effects. Judged from the data (Table 2), there were no consistent effects with treatment or time, suggesting a very high stability of nitramines. The fact that only some 25 % of nominal concentrations were detected by the analysis is troublesome however, and may suggest absorption in the resin column or other analytical artefacts,

and we thus strongly recommend following up analysis before arriving at final conclusions. Due to analytical problems only one of the two nitramine species has also so far been analysed.

Table 2. DMA-NO_2 concentrations at day 0 (start), day 1 and day 28 (final) of all combinations of DOC as mg C l^{-1} and TP as µg P l^{-1} for each temperature (5 ^{o}C and 15 ^{o}C). The "nd" denotes "not detected" and "*" denote problems with the analysis.

			Day 0	Day 1	Day 28
Temp., °C	**mg C l^{-1}**	**µg P $^{-1}$**	**µg DMA-NO_2 l^{-1}**	**µg DMA-NO_2 l^{-1}**	**µg DMA-NO_2 l^{-1}**
5	0	0	-	93	99
5	10	0	-	70	138
5	20	0	-	136	125
5	0	25	-	118	140
5	10	25	-	75	121
5	20	25	-	98	139
5	0	50	-	92	143
5	10	50	-	83	126
5	20	50	-	98	105
15	0	0	*	116	160
15	10	0	139	125	151
15	20	0	105	*	130
15	0	25	112	92	39
15	10	25	111	124	nd
15	20	25	114	118	nd
15	0	50	116	114	nd
15	10	50	105	150	nd
15	20	50	*	91	nd

The effects of Dimethylnitramine (DMA-NO_2) on phytoplankton *Selenastrium capricornutum* and zooplankton; *Daphnia pulex* and *Daphnia magna*.

As an initial screening of the potential impact of nitramine compounds on the environment and the potential effects on the biota, we run a pilot toxicity test on survival (Test 1) and life history (Test 2 and Test 3) of plankton. A review of the toxicity of compounds that will be potentially emitted to the environment by the process of CO_2 capture using selected amine compounds (MEA, MDEA, AMP, PIPA). The main secondary products include amides (formamide and acetamide), nitrosamines and nitramines have been conducted by Norwegian Institute for Water research (NIVA) (Brooks 2008), mostly based on chronic exposure to fish and invertebrates. A potentially harmful level was estimated above 0.2 µg/L of nitramine, yet there may be strong variability in toxisity of various nitramines, also with respect to ambient conditions and tested organism. Also strong differences should be expected between acute and more chronical exposure where life cycle attributes of the tested organisms would be relevant. The substance tested in our preliminary assay was also not included in the cited review. In fact there is a general lack of toxisity data for nitramines, hence amines have been applied as a proxy in acute toxisity tests where the acute toxicity freshwater invertebrates is shown in yielded LC_{50} concentrations between 10 and 250 mg/L, with the most toxic effectfound in the crustacean zooplankton *Daphnia magna* when exposed to PIPA. Corresponding LC_{50} for algae and bacteria ranged between 6 and 733 mg/L, with bacteria as the most sensitive (Brooks 2008).

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Test 1: Toxicity of Dimethylnitramine (DMA-NO_2) on phytoplankton; *Selenastrium capricornutum*.

Methods

To test the toxicity of Dimethylnitramine (DMA-NO_2) on phytoplankton, we use the green, unicellular algae *Selenastrium capricornutum*, a green algae that is kept in culture at Centre of Ecological and Evolutionary Synthesis (CEES) at the University of Oslo. *Selenastrum* was grown at standard freshwater medium COMBO (Kilham et. al., 1998). The experiment was performed by adding 20 ml of diluted algae culture into 20 ml glass vials containing five different concentrations of nitramines (0, 5, 50, 500 and 5000 µg l^{-1}). The vials were placed on a shaking table and exposed for 70 µmol light. The optical density (OD) at 663 nm was measured by using a Shimadzu UV-160A photometer. The OD for each sample was measured over a period of two weeks.

Results

The effects of the freshwater chlorophyte *Selenastrum* (phytoplankton) revealed no effects judged as accumulated biomass (and thus growth rate) for concentrations up to 500 µg/L over a 2 weeks incubation period, while at 5000 µg/L, a slight lower final biomass was observed (Fig. 1).

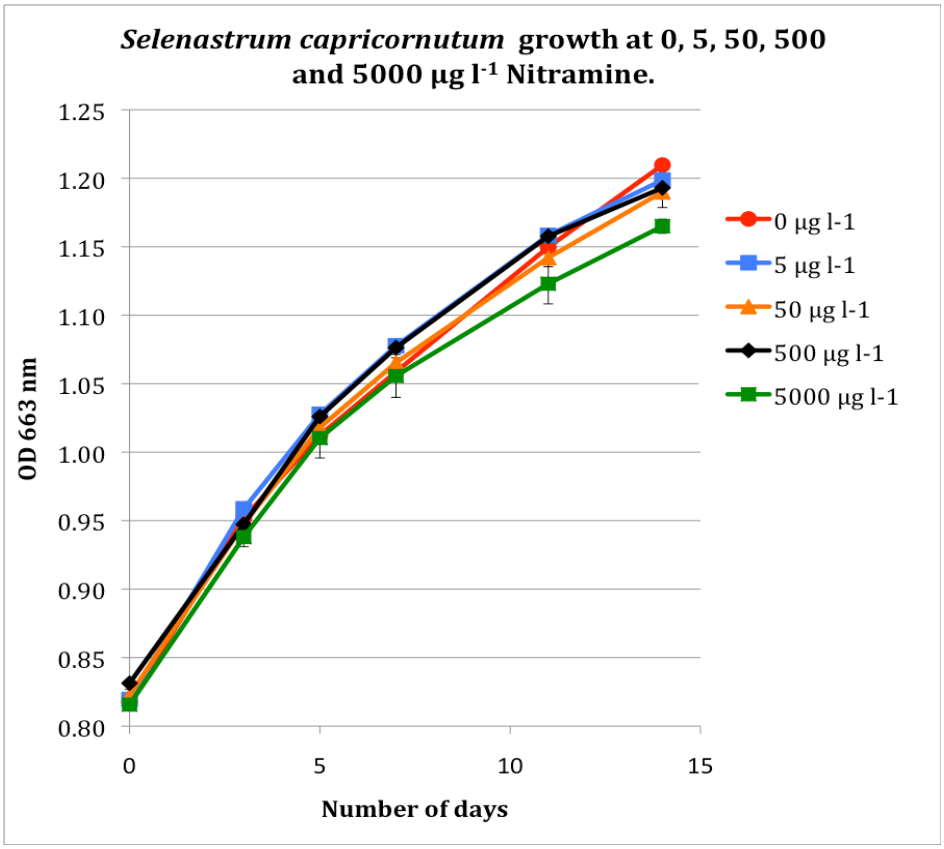

Figure 1. The development of the green algae selenastrum capricornutum at day 0, 3, 5, 7, 10, 11 and 14 at 4 different DMA-NO_2 (dimetylnitramine) concentrations (0, 5, 50, 500 and 5000 µg

l^{-1})

Test 2: Effects of Dimethylnitramine (DMA-NO_2) to growth and reproduction of zooplankton; *Daphnia pulex*.

Methods

The pre-experiment was performed in 5 glass beakers (600 ml) containing 400 ml ADaM culture medium design for culturing of zooplankton. To each of the glass beaker was added DMA-NO_2 to final concentration gradient of 0, 5, 50 500 and 5000 µg l^{-1}. 20 *Daphnia pulex*, not older than 48 hours, were placed in each glass beaker. The Daphnids originated from a stock culture grown at the Centre of Ecological and Evolutionary Synthesis (CEES) at the University of Oslo.

The algae (*Selenastrum capricornutum*) was cultured on COMBO culture medium and used as food for the Daphnia. Selenastrum was centrifuged before adding to reduce mixing of the to culture medium; 7 Eppendorf tubes were filled with 1 ml of algae for every concentration (7x5 tubes). These tubes were centrifuged 2 minutes by 2000 rpm. The surplus COMBO was removed with a pipette and the nearly pure algae was rinsed to the solutions. Afterwards, the Daphnids were feed every third day with 7 ml algae per beaker and their medium was

changed once a week. The Daphnids was observed over a period of two weeks and their response was simply assessed by number of individuals present along the gradient of nitramine concentrations. For the *Daphnia pulex* experiments, both survival and reproduction was tested, while for *Daphnia magna*, we only tested crude survival over time.

Results

The results are displayed in Figures 2, 3 and 4. By and large, there were no effects for concentrations up to 500 µg/L, while there seemed to be an almost complete mortality at the highest concentration (5000 µg/L).

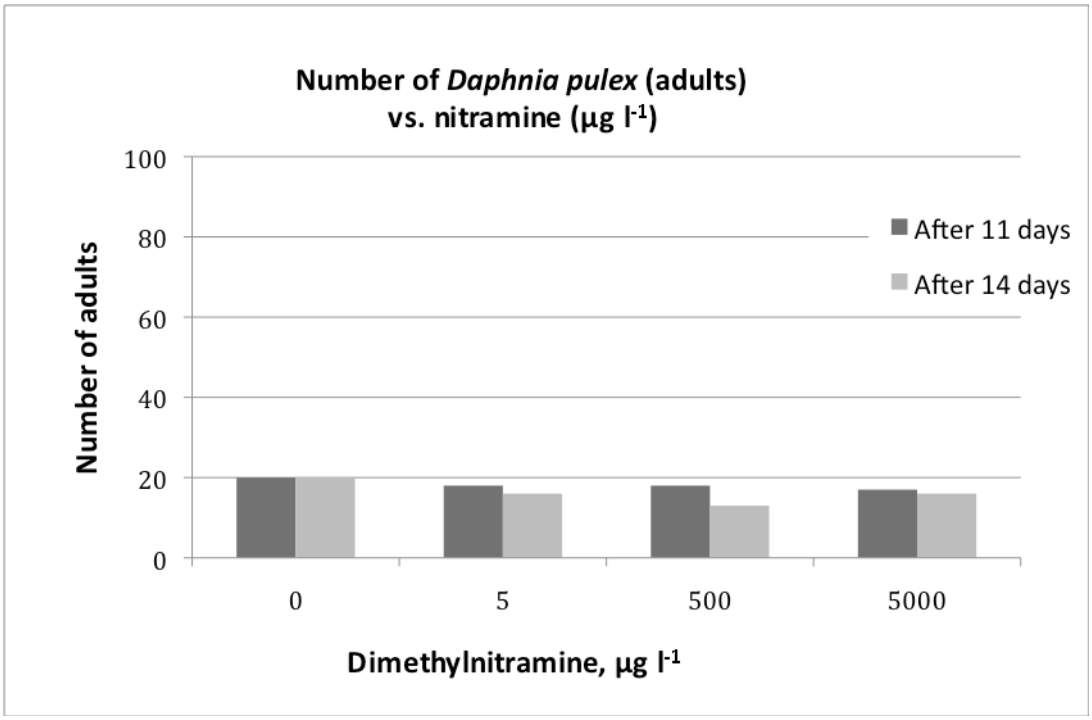

Figure 2. The number of adults *Daphnia pulex* alive after 11 days and after 14 days in 4 different concentrations of DMA-NO_2 (0, 5, 500 and 5000 µg l^{-1}), the number of juveniles and the juveniles per clutch after 11 and 14 days.

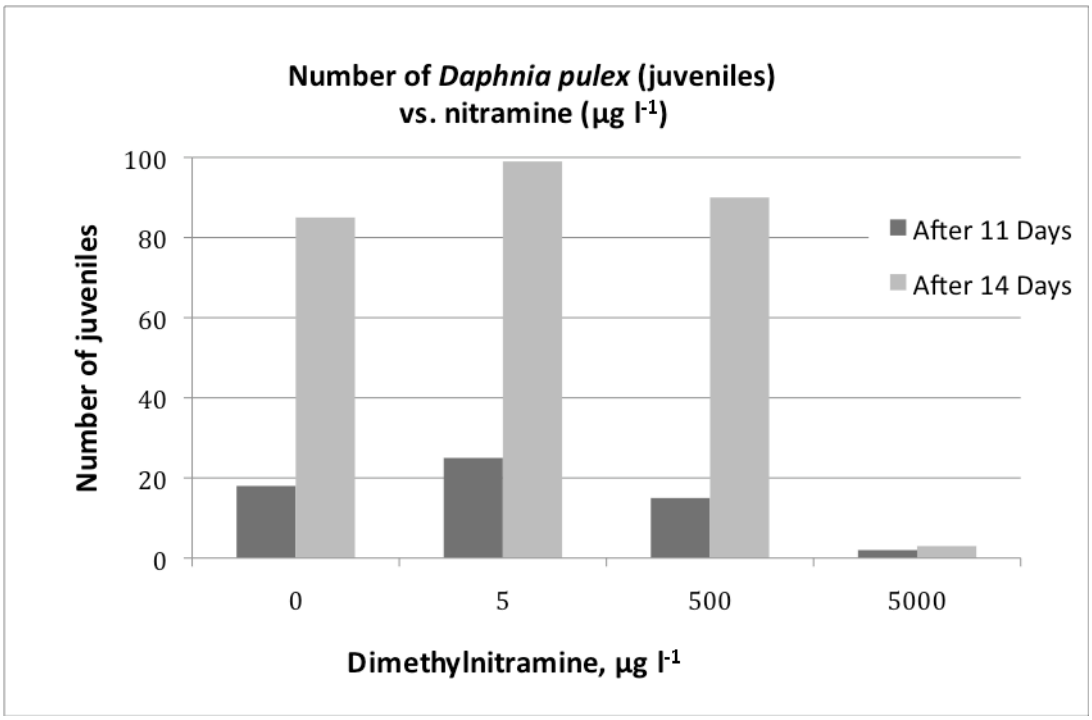

Figure 3. The number of juvenile *Daphnia pulex* alive after 11 days and after 14 days in 4 different concentrations of DMA-NO_2 (0, 5, 500 and 5000 µg l^{-1}), the number of juveniles and the juveniles per clutch after 11 and 14 days.

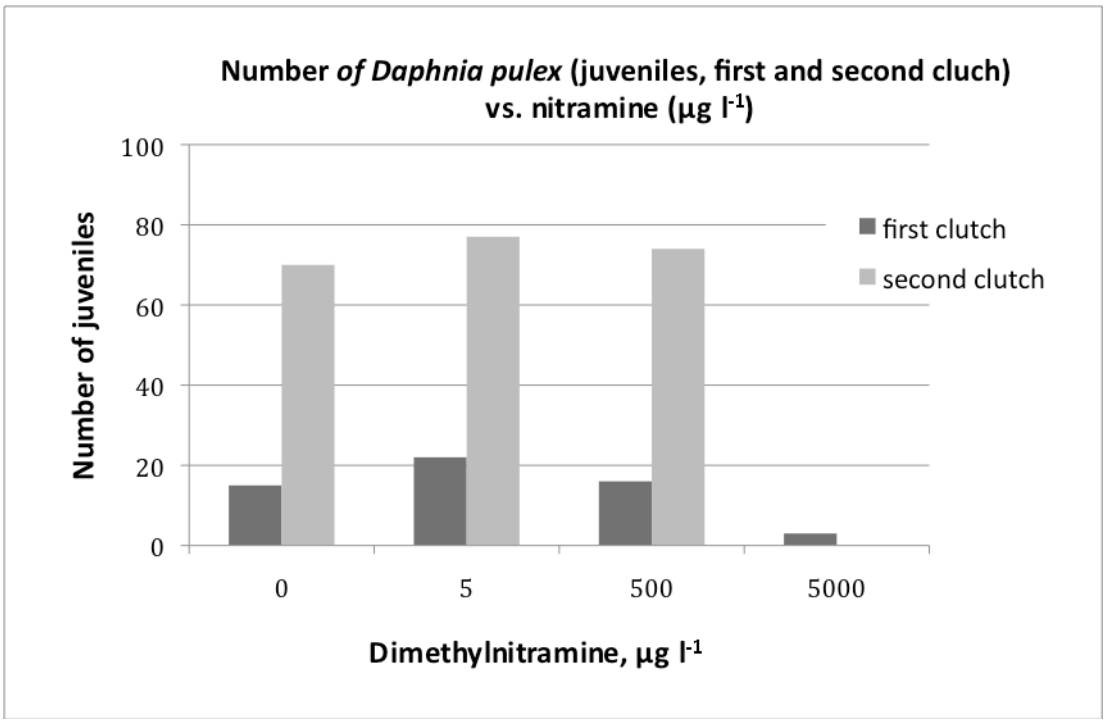

Figure 4. The total number of offspring from first and second clutch of *Daphnia pulex* alive after 14 days in 4 different concentrations of DMA-NO_2 (0, 5, 500 and 5000 µg l^{-1}).

Test 3: Effects of dimeythylnitramine (DMA-NO_2) on *Daphnia magna*.

Methods

The experiment was run in glass beakers (80 ml) with five *Daphnia magna* and performed in triplicates. The amount of ADAM medium was increased in comparison to Experiment 1 to avoid density stress. The Daphnids were juveniles from a stock culture from the Centre of Ecological and Evolutionary Synthesis (CEES) at the University of Oslo. The juveniles were also freshly hatched and not older than 48 hours. The solutions of nitramines were prepared with a nitramine stock solution of 200 mg l^{-1} in ADAM culture medium. Following DMA-NO_2 concentrations were chosen to test the toxicity; 0, 5, 50, 500 and 5000µg l^{-1}.

Solutions were prepared first and Daphnids were added afterwards to avoid a shock caused by the addition of the nitramines. Beakers were placed on a plate and moved to an air conditioning room (10°C). Effects were observed over a period of 48 hours.

Results

There was a decreased number of survivors with increased concentrations of DMA-NO_2 (Fig. 5), except for the highest concentration, where there however were clear signs of decreased movement of the daphnids which could result in reduced ventilation rate at the highest concentration.

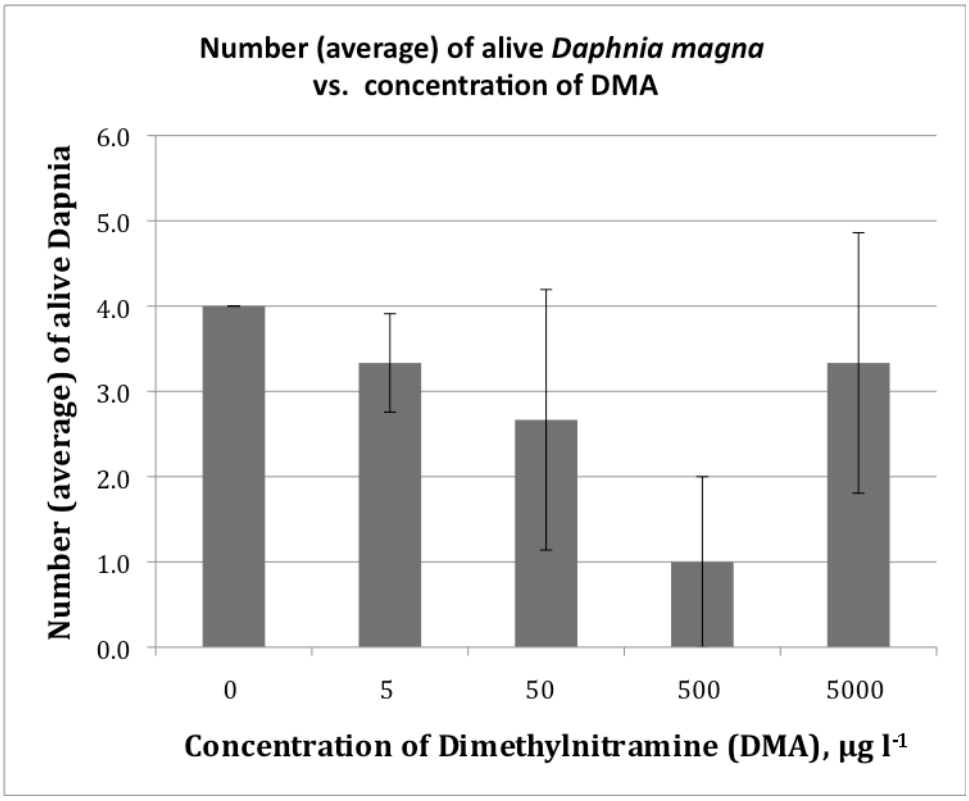

Figure 5. The number (average ± standard deviation) of living *Daphnia magna* after 14 days kept in 5 different concentrations of DMA-NO_2.

Conclusions:

These initial screenings should be seen as rather preliminary, yet with a possible exception for *Daphnia magna*, they suggest no harmful, short-term impact of DMA-NO_2 concentrations < 5000 µg l^{-1}. This seem to be below the level that generally is expected to be harmful for aquatic organisms under acute exposure, while it nevertheless may impose megative effects under more chronic exposure (Brooks 2008). Moreover these data need also to be seen in context with the range of concentrations that may be expevted under natural conditions, i.e. in the proximity of CO2 treatment plants, which again will depend on a range of parameters (cf. Shao and Stangeland 2009). The rate of degradation of DMA-NO_2 seems to be slow however, yet following-up experiments with better analytical resolution and more species of DMA-NO_2 should be performed before arriving at final conclusions. We also strongly recommend sampling from natural aquatic localities in the vicinity of CO_2-treatment plants to reveal the levels and concentrations that could be anticipated. Following-up toxicological tests should be more sophisticated than those presented here, and also then relate to concentrations measured *in situ*. Moreover we recommend that assays should include studies on microbial communities, where respiratory assays on natural bacterial communities could serve as a good proxy of metabolic responses on natural systems.

Reference:

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Klüttgen, B., Dülmer, U., Engels, M. and Ratte, H.T. 1994. ADaM, an artificial freshwater for the culture of zooplankton. Wat. Res. Vol. 28, No. 3 pp. 743-746.

Shao, R. and Stangeland, A. 2009. Amines used in CO_2 capture – health and environmental impacts. Bellona Report.