Project information

Project title

The combined effects of radionuclides, metals and organic contaminants in produced water on early life stages of Calanus finmarchicus

Year

2012/2013

Project leader

Louise K. Jensen, NRPA

Participants

- Louise Kiel Jensen, Norwegian Radiation Protection Authority
- Steven Brooks, Norwegian Institute for Water Research
- Jasmine Nahrgang, University of Tromsø

Flagship

Hazardous substances, Theme: Petroleum pollution

Funding Source

Fram Centre

Summary of Results

The main objective of this project was to evaluate the relative toxicity of the different contaminant categories found in produced water on the early life stages of copepods. The different contaminant categories includes Artificial Produced Water (APW) representing the organic fraction, radionuclides (here radium-226), metals (here barium) and the added scale inhibitor SI-4470.

Originally, the sub-Arctic copepod species Calanus finmarchicus was selected as the target species due to their size and their importance in the marine food web. However, as they only reproduce once a year at higher latitudes and after the experiment was delayed to after their main spawning period for various reasons, the smaller Acartia longiremis was chosen as a substitute.

Stock solutions for the APW were prepared by NIVA following the mixture of Holth et al 2008. Concentrated stock solutions were prepared in acetone stored at -20oC. When required the stock solutions were defrosted and diluted as appropriate with filtered seawater (0.45 µm) before use. Radium, barium and a scale inhibitor was added to filtered sea water and mixed. In addition, samples for concentration confirmation of the APW and barium were taken and currently being analyzed by NIVA.

Exposure studies were performed at the University to Tromsø in a cold chamber. The design was semi-static with water exchanges 3 times a week. Exposures were done in the dark.

Preliminary, a series of exposures were performed on the eggs of A. longiremis to determine an exposure concentration of all treatments that allowed for the highest possible hatching success. Based on these pilot experiments it was determined to keep the exposure concentrations in the nauplii growth experiment (see later) at 25 % of average discharged concentration of the various chemicals. However, radium-226 concentration was kept at the concentration reported for discharges in the North Sea.

In the nauplii growth experiment chemical exposure of eggs and hatched nauplii continued until 100 % mortality was recorded in all replicates. Water was changed every 2-3 days and live nauplii were counted and transferred to fresh exposure media. From day 5 algae food was provided at 1000 cells ml-1 upon water exchanges.

Hatching success was recorded after three days and ranged from 50-64 %, with the control treatment yielding the lowest success and the radium exposure generating the highest hatching success (see Fig. 1). The mortality of the nauplii was assessed in based on number of live nauplii on August 29th. In the APW and the scale inhibitor treatments 100 % mortality was recorded after 5 days (see Fig. 2) whereas in the treatment consisting of all exposure categories in combination 100 % mortality was found on day 7. In the barium and the radium treatments, specimens survived until day 14, and the experiment was terminated on day 16 with still a very few control specimens alive (Fig 2).

The results shown here reveal various issues:

- Concentrations of chemicals not inhibiting the hatching of eggs may cause mortality on early life stages in copepods.
- The various categories of chemicals in produced water show different toxicity towards early life stages with APW, Scale inhibitor and “all in combination” exceeding the toxicity of radium and barium.
- Given the high mortality in the control treatments, raising nauplii in small quantities of water with regular disturbances, is a large challenge.

The last point listed above is vital for further work on this project. The transition to nauplii stage III (NII)(the first feeding stage) is considered a threshold in their development. Failure to raise nauplii above stage NII in smaller quantities of water has been observed also in other experiments (Grote and Halvorsen, Uit, pers med.) and published work on nauplii development often have NIII as the starting

stage.

Based on the experience achieved during this pilot experiment we hope to develop the project further;

- The timing of the experiment will be coordinated with the reproduction timing in C. finmarchicus, ensuring that we use the larger species in our experiments.
- Larger, non-invasive, experimental chambers will be used and a backup culture of nauplii in large containers will ensure continuation also of the larger nauplii stages.
- Treatments will be subsampled and microscope pictures will be taken of all specimens to correctly determine stage development.
- Dose response curves should be determined for each chemical category.
- Mixture exposures of selected candidates to evaluate their effects interaction (CA/IA) according to the mixtox-model will be run.

Holth, Tor Fredrik; Nourizadeh-Lillabadi, R.; Blæsbjerg, M.; Holbech, Henrik; Petersen, G.; Alestrøm, P. & Hylland, Ketil (2008). Differential gene expression and biomarkers in zebrafish (Danio rerio) following exposure to produced water components. Aquatic Toxicology. ISSN 0166-445X. 90(4), s 277- 294

Published Results/Planned Publications

At the current stage of the project, the results acquired do not seem complete enough to justify a scientific publication.

Communicated Results

The project will be presented on the web pages of Norwegian Radiation Protection Authority under the heading "research" hopefully during winter 2012/2013.

A presentation of the work will be given at an Apecs Webinar on November 22nd, 2012. This webinar is allocated to the Hazardous Substances Flagship, and one talk will mainly be on this project.

Interdisciplinary Cooperation

On the original proposal, Carmen Martinez from Uit was included, as she is experienced in metal toxicity. Unfortunately, she moved before the experiments were conducted and we lost a strong resource in the project. However, with a background on experimental biology, physiology and ecotoxicology and the chemical aspects of exposure studies the participants have complemented each other in completing the first part of the project.

Budget in accordance to results

This was a pilot study to test the feasibility of using earlier stages in exposure studies and to build up a test system that would work over longer exposure times. Valuable experience is gained and we seek to develop the experimental setup further. In addition, important equipment has been acquired and is stored for use for further experiments.

Could results from the project be subject for any commercial utilization

No

Conclusions

a) As described in section 5, we wish to further develop the project based on the experience acquired in this pilot project. The preliminary results indicate that the different categories of contaminants in produced water have different toxicity on early life stages of copepods. However, further experiments are required to fully evaluate their effects interactions.

b) The pilot experiment revealed that manual handling of nauplii probably disturbs their development. Originally, it was planned to use large non-invasive experimental chambers but due to a change in species these was not applied. In future experiments these chambers (which we have in store) will be tested to evaluate if the development of the copepods can be maintained from eggs to copepodites (later larvae stages) in an experimental setup.