

# “Long-term (chronic) effects of produced water effluents affecting reproduction in marine crustacean plankton”.

*An initiative to meet the need for parameterized data from ecologically relevant species in modelling fate and impact of petrogenic oil emissions to sea.*

Organized as a consortium with members from NTNU, BioTrix, SINTEF, and Bodø University College.

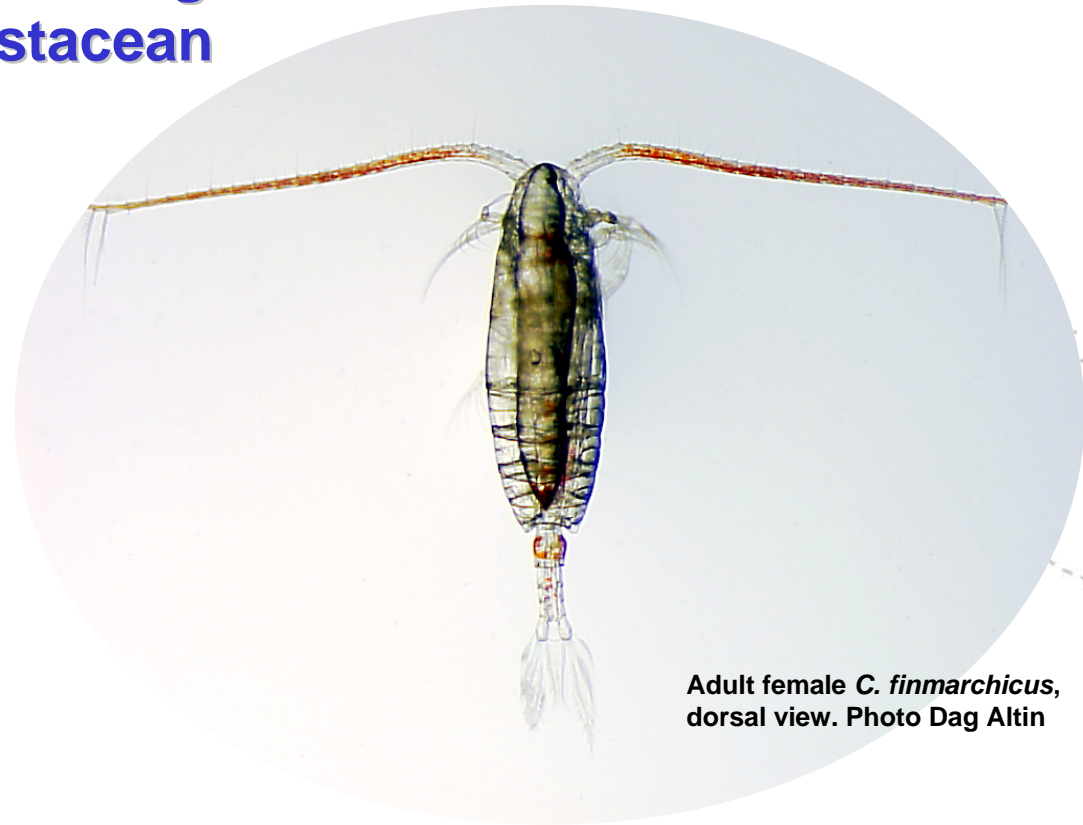


BioTrix



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Adult female *C. finmarchicus*, dorsal view. Photo Dag Altin



## Project organization and cost

- *2001 -2009. Entire project period. NFR PROFO - PROOF - PROOFNY*
- *2001: Pre-project. NFR PROFO*
- *2003-2005: Main project phase 1: “Establishment of Calanus finmarchicus laboratory cultures conditioned for exposure studies”. NFR PROOF*
- *2005-2009: Main project phase 2: “Exposure methodology development and exposure studies”. NFR PROOF - PROOF-NY*
- *Overall cost frame: In excess of KNOK 9000*

## Main aims

- *Establish novel insight into the susceptibility of pelagic crustacean plankton (viz. copepods) in northern seas for acute and chronic exposures to oil-related hydrocarbons, with special emphasis on *C. finmarchicus*. Reveal secondary impacts on other species or ecosystem structures induced by- or related to biological effects on the plankton level.*
- *Derive key input data to existing and future computer-based simulation tools to increase their accuracy and predictive power.*
- *General competence build-up within the field of marine ecotoxicology. Strengthening of regional competence in marine ecotoxicology during a strategic co-operation between NTNU, SINTEF, Bodø University College and BioTrix*

# 4 Main frames for the project

- *Establishment of methodology for laboratory simulation studies of effects of petrogenic oil-in-water residuals on development and reproduction in marine zooplankton.*
- *Establishment of multi-generation laboratory cultures of *Calanus finmarchicus*, and development of the necessary methodology.*
- *Development and implementation of methodology for oil droplet suspension formation.*
- *(Establishment of logistics and methodology for experiments with real Produced Water (PW) from one or more production platforms)\*.*
- *A number of acute tests (maximum 96h) to reveal sensitivity ranges.*
- *A number of long-range tests (4 weeks or more) with constant exposure to low concentrations*

*\* For now omitted*

## 5 Main frames for the project

- *A number of acute tests (a number of days, generally higher concentrations) with following registration of long-range effects*
- *Miscellaneous tests to reveal specific effects.*
- *Evaluation of the results with regard to their applicability as input parameters for fate and impact modelling, and consequences for plankton community or the entire pelagic ecosystem in the actual recipient.*

# Main species target

- *The main target for the investigations is the calanoid copepod Calanus finmarchicus.*
- *C. finmarchicus constitute a significant biologic element of the pelagic community of the Norwegian sea and North Atlantic.*
- *C. finmarchicus forms a crucial trophic link between primary production and secondary consumers in the North Atlantic.*
- *The ecology of C. finmarchicus is fairly well surveyed due to a comprehensive field research activity over a number of years.*
- *C. finmarchicus accumulates large amounts of fat, mainly in the form of wax esters. Hence the species has a pronounced tendency to absorb lipophilic components from the water, including oil components. Such components may be transferred to higher trophic levels along the food chain .*



Eggs



Photo Tora Bardal

Nauplii



Photo Dag Altin

Copepodid V



Photo Dag Altin

Adult female

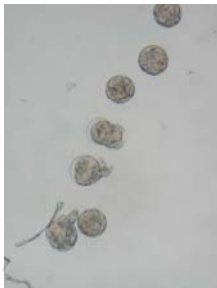


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## Main species target

- *Information on long-range vulnerability of C. finmarchicus to low-concentrated xenobiotics in the water has been, and is still, scanty, mainly due to the inherent difficulties in establishing firm exposure/effect connections under fluctuating field conditions.*
- *Many earlier attempts to cultivate the species have failed. Few if any of the plankton species now used for ecotoxicity testing represent a good model for C. finmarchicus.*
- *C. finmarchicus is currently considered a very relevant candidate for commercial exploitation, and technology for catching and processing is under development.*



Eggs



Photo Tora Bardal

Nauplii



Photo Dag Altin

Copepodid V

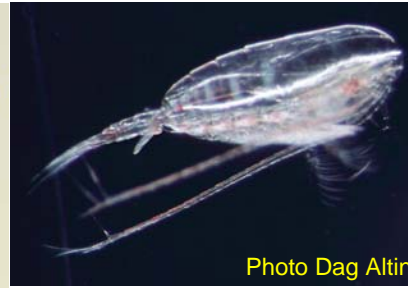


Photo Dag Altin

Adult female



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# Current outcome status

## *Main outcomes pre-project:*

- *A literature study*
- *Invention of techniques for collecting *C. finmarchicus* alive (from sea)*
- *Testing of micro algae species for suitability as food for *C. finmarchicus**

## *Main outcomes main project phase 1 were:*

- *Viable cultures of *C. finmarchicus**
- *Development of prototypes of several exposure set-ups*
- *Establishment of methodology for a number of exposure effect endpoint*

## *Main outcomes main project phase 2 were:*

- *Establishment and testing of several exposure set-ups*
- *Establishment and testing of novel methodology for oil droplet suspension formation*
- *Establishment of systems for non-damaging registration of development status*
- *Acute and long-term tests with WSF/WAF and oil droplet suspensions*



# The cultures of *C. finmarchicus*

- *Currently maintained for almost four years and 19 generations*
- *Reared in flowing and filtered seawater*
- *Diet is a mixture of three micro-algae; *Isochrysis galbana*, *Dunaliella tertiolecta*, and *Rhodomonas baltica**
- *Fouling and infection is low*
- *Body size comparable to wild individuals*
- *Fat deposition as in wild individuals or better*



Photo Arne Kjøsnes



Photo Arne Kjøsnes

Production of  
feed algae

280L polyester  
rearing tanks

# Selected endpoints

## Development

- Stage-specific development time
- Stage-specific mortality

## Biometric measures

- A standard scheme has been established.

## Energy stores/reserves

- Fat deposition and fat composition
- Adenylate charge (high-energy phosphates)

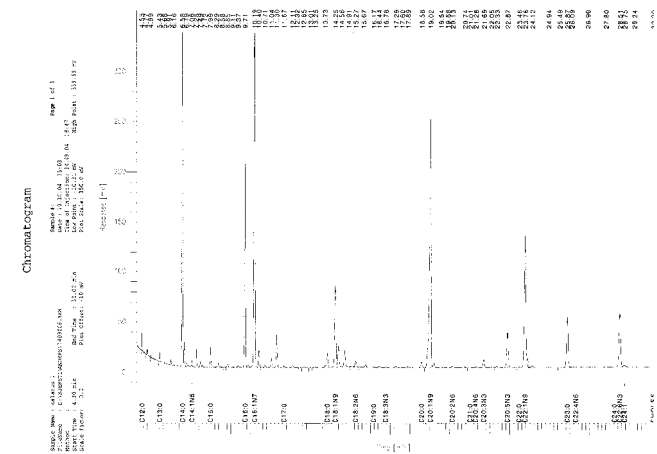
## Reproductive deficiencies

- Impaired gonad development
- Egg production
- Hatching rate

## Gene expressions and other molecular endpoints



Scheme for biometric measurements



GC FID chromatogram of fatty acids from a single female *C. finmarchicus*

## Exposure equipment

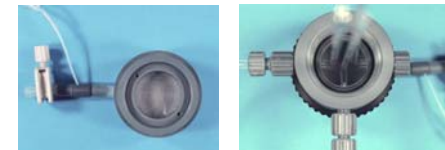
*Generally custom-made. Design depends on experiment layout, size of the animals (e.g. nauplii or adults), or the exposure medium (e.g. water with oil suspension, or dissolved components only). A system for long-range (chronic) exposure to sea water with dissolved chemical components is shown below*



Photo Arne Kjesnes



Photo Dag Altn



**Above: A single exposure chamber. Left: A complete set-up**



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## Goal achievement

*The project seems to reach its main aims reasonably well. New insight is gathered into the susceptibility of pelagic crustacean plankton in northern seas for acute and chronic exposures to oil-related hydrocarbons. Input data for simulation tools are now appearing. And the project has indeed strengthened the competence in marine ecotoxicology.*

*To highlight, some presumably challenging tasks have been successfully accomplished:*

- *Establishment of multi-generation cultures of *C. finmarchicus**
- *Development of working equipment for long-range flow-through exposure experiments with copepods*
- *Development and implementation of methodology for controlled and on-line production of oil droplet suspensions*
- *Development and implementation of an alternative methodology for online WSF generation*

*Additional methodology has also been invented, e.g. the system for un-lethal microscopic inspection of individuals by the aid of a specially designed flow-cell.*

*Most proposed endpoints are still considered, but additional molecular and genetic endpoints have been added.*

# Goal achievement

*An example of the implementation of new methods is the fluorescence verification of oil droplet exposure as shown below:*

- A: Size distribution of algae in exposure media*
- B: Size distribution of oil droplets in exposure media*
- C: Exposure media showing feed algae in red, oil droplets in yellow*
- D-E: Micrograph of C. finmarchicus after high exposure, D without-, E with fluorescence*
- F-G: Micrograph of C. finmarchicus after low exposure, F without-, G with fluorescence*

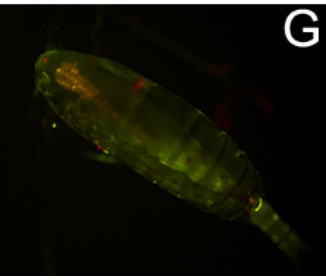
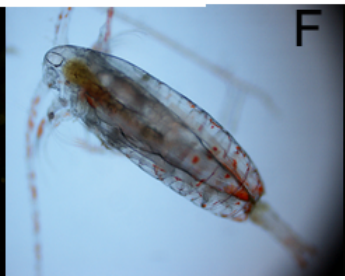
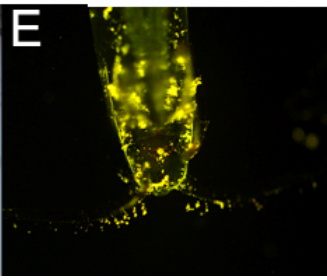
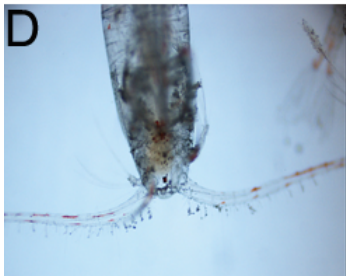
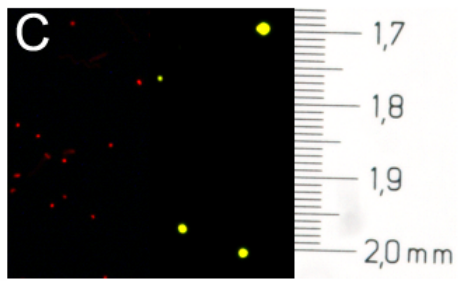
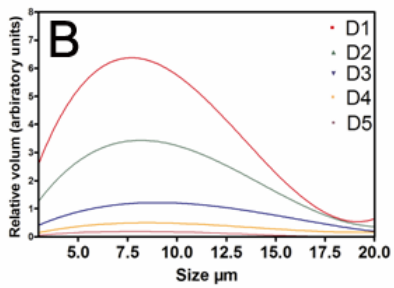
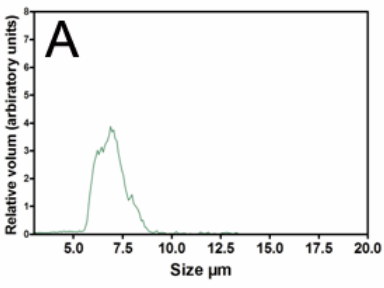


Photo Dag Altin

## New and relevant ecotoxicological tests

*The multi-generation cultures of *C. finmarchicus* and development of dedicated exposure equipment have given the possibility to establish relevant long-range standard ecotoxicological tests protocols based on this species.*

*From our point of view there is especially two exposure procedures that should be given attention. A version of each are already established within the project, and may relatively easily be adapted to standard tests.*

- 1) *A development test, following *C. finmarchicus* from hatching to almost adult stage (e.g. 4 weeks) under continuous exposure to low concentrations. As now developed, the test is suitable for solved components only (including WSF), but may be change to accept exposure to oil droplet suspensions through defined periods.*
- 2) *An acute exposure – long-range effect test where specimens of a specific age (e.g. copepodid V with large fat stores) are exposed to relatively high concentrations of solved components or oil droplet suspensions. Then the cohort is followed in clean water through further development and maturation until egg-laying, and the effect on egg-laying frequency and egg hatching success recorded.*

## Acknowledgements

*We are grateful to the Research Council for having funded the project and hence made the reported achievements possible.*

*We would also want to thank StatoilHydro and the other companies in OLF (oljeindustriens landsforening) who have indirectly funded more than half of the budget through their agreement with the Research Council (PROOF and PROOF-NY).*

*The technical staff of our institutions has offered the project their full support, and many colleges have helped through useful discussions. We are very grateful to them all.*

# NTNU Sealab

*NTNU's new facility in Trondheim for marine experimental research. Constant supply of good-quality seawater for from large depths in the fjord – for experiments and animal rearing. Data - controlled conditioning rooms for experiments. Up to date analytical laboratories with necessary instrumentation.*



Photo Erik Hey

*Sealab (under construction) with NTNU's research vessel Gunnerus in the foreground.*

