Ecotoxicological testing of marine petrogenic emissions using laboratory-raised stocks of *Calanus finmarchicus* as model target.

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

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

Rearing methodology and test set-ups have been developed by a research group with members from NTNU, BioTrix, SINTEF, and Bodø University College.

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The rationale behind choosing *C.finmarchicus* as a model target for ecotoxicity testing of petrogenic emissions to the sea water column

C. finmarchicus constitute a significant biologic element of the pelagic community of the Norwegian sea and North Atlantic. Standing biomass has been estimated to be more than 200 mill. tons and annual production up to 300 mill tons. In some areas the species may constitute up to 90% of standing zooplankton biomass.

C. finmarchicus forms a crucial trophic link between primary production and secondary consumers. Important consumers include commercial important fish species as cod and herring. A significant decline in production or standing biomass of C. finmarchicus is considered to have a marked effect on several fish stocks.

The ecology 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 absorbe lipophilic components from the water, including oil components. Such components may be transferred to higher trophic levels along the food chain .

Few if any of the plankton species now used for ecotoxicity testing represent a good model for C. finmarchicus. A common used species as Acartia tonsa is, for instance, very much smaller, and does not accumulate lipids to a comparable level.

C. finmarchicus is now considered a very relevant candidate for commercial exploitation, and technology for catching and processing is under development. Detailed information on the biology of the species and its vulnerability to pollution should therefore be demanded.

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"Long-term (chronic) effects of produced water effluents affecting reproduction in marine crustacean plankton"

This NFR-funded project series made it feasible to make C. finmarchicus available for routinely ecotoxicity testing and mechanistic experiments. The project provided the economic and hence infrastructural basis for successful establishment of reproducing laboratory cultures, which in turn is a prerequisite for detailed investigations on the species

Total cost frame over the entire project period is more than NOK 9 mill.

The project series was commenced in the form of a pre-project, approved by Research Council in the spring of 2001, and accomplished during the autumn the same year. Main outcomes were:

- *A literature study*
- *Invention of techniques for live sampling of C. finmarchicus,*
- *Testing of micro algae species for suitability as food for C. finmarchicus*

Phase 1 of the main project, "Establishment of Calanus finmarchicus laboratory cultures conditioned for exposure studies", was approved by the Research Council in the spring of 2003, and finished early in 2005. Main outcomes were:

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

Phase 2 of the main project, "Exposure methodology development and exposure studies", was approved by the Research Council in 2005. Project closing time is ultimo Mars 2009. Main outcomes so far have been:

- *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*
- *Some tests with WSF/WAF and oil droplet suspensions*

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The cultures of *C.finmarchicus*

Some culture characteristics:

- *Maintained for more than three years and 15 generations*
- *Reared in flowing and filtered seawater*
- *Diet is a mixture of three micro-algae; Isochrysis galbana, Dunaliella terticolecta, and Rhodomonas baltica*
- *Sufficient number of males to secure high reproduction*
- *Fouling and infection is low*
- *Body size comparable to wild individuals*
- *Fat deposition as in wild individuals or better*

Production of feed algae

Photo Arne Kjørsvik

280L polyester rearing tanks

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Life cycle and development

Number of stages

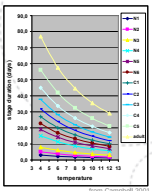
- *egg*
- *nauplii* — *6 stages*
- *copepodid* — *5 stages*
- *adult males and females*

Mating and fertilization

- *sperm is produced in a spermatophore which is attached to the female*
- *the sperm moves from the spermatophore into a spermatheca in the female body*
- *the eggs are fertilized when they are mature and ready for being laid*

Development

- *egg:* — *temperature dependent*
- *N1-N2 (non-feeding):* — *temperature dependent*
- *N3-N6:* — *temperature and food dependent*
- *C1-C5:* — *temperature and food dependent*



from Campbell 2001

Development temperature dependency

Approximate development non-limiting food density >100 µg C L^{-1}

Approximate growth non-limiting food density >100 µg C L^{-1} for nauplii >120 µg C L^{-1} for copepodids

from Campbell 2001

Eggs

Photo Dag Altin

Newly hatch nauplii

Photo Dag Altin

Large copepodid with big fat sac

Photo Dag Altin

Adult female with spermatophore attached

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Experiments on reared *C. finmarchicus*

In general:

- *Controlled experiments may be performed whenever during the year, on un-damaged specimens with known history and status*

Exposure simulations:

- *Acute exposures may be done on whatever developmental stage*
- *Long-range exposures may be performed across the whole life span of the species, or across defined developmental periods*
- *Specimens reared for test purposes may be adjusted with regard to nutritional status or other parameters prior experiments, e.g. to investigate the effect of feed quality or quantity on their ability to accumulate petrogenic oil components or resistance against chemical stress*

Suitability for standard eco-toxicological testing:

- *Standard/commercial ecotoxicological tests based on C.finmarchicus could be highly ecologically relevant, but depend on the availability of reared stocks with defined status*

Adaption to laboratory conditions:

- *The cultured C. finmarchicus now seems well fitted to laboratory conditions, especially concerning behaviour. This may be indicative of a genetic adaptation to laboratory conditions. This, in turn, should be considered when using the material for specific purposes.*
- *There is no measured deviations in biometry between reared and wild specimens*
- *There is currently no reason to suggest a significant change in chemical sensitivity in reared species. We will, however, perform tests to confirm this assumption*

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Exposure equipment for petrogenic oil suspensions or water extracts

Several exposure systems have been constructed. Systems are generally custom-made, and the design depends on experiment layout (e.g. few or many individuals), the size of the animals (e.g. nauplii or adults), or the medium (e.g. water with oil suspension, or dissolved components only). As an example, a system for long-range (chronic) exposure to sea water with dissolved chemical components is shown below

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

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Endpoints

A number of endpoints are addressed, of which some are presented below. Selected endpoints should preferably give information about impacts on biological functions of high adaptive value to the species, to reveal possible consequences on population level.

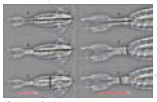
Development
- *Stage-specific development time*
- *Stage-specific mortality*

Biometric measures
- *A standard scheme has been establishet*
- *Indicative of malfunction in food assimilation ability or development*

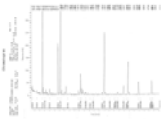
Energy stores/reserves
- *Fat deposition and fat composition*
- *Adenylate charge (high-energy phosphates)*

Reproductive deficiencies
- *Impaired gonad development*
- *Egg production*
- *Hatching rate*

Gene expressions and other molecular endpoints
- *(This topic will be covered by Dr. Bjørn Henrik Hansen in the next presentation)*



Scheme for biometric measurements



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

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Invitation to co-operation

We do think the establishment of the C. finmarchicus cultures at SINTEF/NTNU Sealab could be of interest for researches also outside our own research group and associates. We would therefore invite to cooperation on research involving mechanistic or other detailed studies on the species. Our research facility is brand new, with state of the art laboratories and supply of good quality seawater from Trondheimsfjorden.

Sealab with NTNU´s research vessel Gunnerus in the foreground.

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