Understanding modes of oil toxicity in the copepod *Calanus finmarchicus*

Bjørn Henrik Hansen, Dag Altin, Trond Nordtug and Anders J. Olsen

SINTEF Materials and Chemistry
Marine Environmental Technology

Effects of oil on copepods

Long-term effects of oil exposure:

- Reduced survival of offspring
- Reduced egg production
- Reduced life length
- Reduced numbers of nauplii produced

Molecular mechanisms underlying these effects have not been revealed

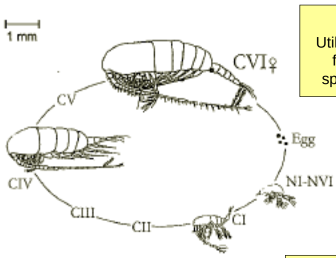
Calanus finmarchicus

Distribution
- Dominating zooplankton species in Norwegian oceans
- 300 million tons annual production
- → Marine ecological key species

Lipid content
- Varies during its life cycle
- Important energy resource for commercial fish species
- Accumulation of lipophilic xenobiotics

SINTEF/NTNU Sealab culture

Life cycle, lipid storage and reproduction



ADULT:
Utilization of lipids for eggs and spermatophores

CIII-CV:
Build-up of lipid storage

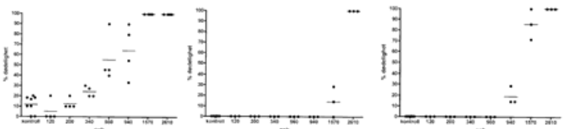
NI-VI & CI-II:
No lipid storage

Is there a relationship between acute toxicity and lipid content?

Stage-specific toxicity testing

Acute toxicity testing (96 hrs) of water soluble fraction (WSF) of 200 ºC+ residue of a North Sea oil on nauplii, copepodites, and adults of *C. finmarchicus*.

NAUPLII: Few % **CV**: 25-50% lipid content **ADULT**: 0-15% lipid content



NAUPLII	CV	ADULT
NOEC: 340 ppb THC	NOEC: 940 ppb THC	NOEC: 560 ppb THC
LOEC: 560 ppb THC	LOEC: 1570 ppb THC	LOEC: 940 ppb THC

Lipid content: CV > adult > nauplii
NOEC/LOEC: CV > adult > nauplii
Sensitivity to oil exposure is dependent on lipid content

*Method adapted from Draft ISO Guideline ISO/DIS 14669, ISO/TC 147/SC5 "Water quality - Determination of acute lethal toxicity to marine copepods (Copepoda, Crustacea)" 1997

Female development and reproduction

FEMALE 1 → FEMALE 2 → FEMALE 3

PHYSIOLOGICAL HIGHLIGHTS

NO EGGS AND HIGH LIPID CONTENT | UTILIZATION OF LIPID RESERVOIR | PRODUCTION OF EGGS | EGG RELEASE AND FERTILIZATION | NO EGGS AND NO LIPIDS

MOLECULAR BIOMARKERS

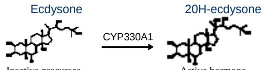
Generation of gene libraries (~6000 expressed sequence tags in GenBank)

Two expressed genes appeared interesting (CYP330A1 and GST):
- Traditionally used as biomarkers
- Putative involvement in vital physiological processes (lipid turnover)

CYP330A1

Ecdysteroid metabolism

- Induced by ecdysone, but not 20H-ecdysone, in shore crabs (Rewitz et al., 2003)

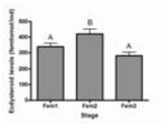


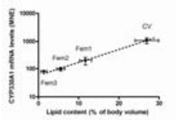
Lipid metabolism

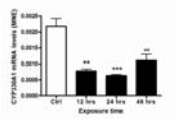
- Expression coincides with lipid storage size in females (Hansen et al., submitted)
  → Fatty acid utilization and egg production

Regulation by xenobiotics

- Induced by B(a)P and phenobarbital in shore crab (Rewitz et al., 2003)
- Reduced transcription following exposure to high naphthalene concentrations (Hansen et al., 2008)







GST

Metabolism of exogenous substrates

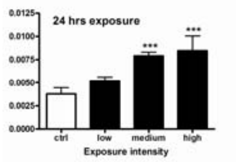
- Biotransformation of xenobiotics (detoxification)
  → GST induction is an exposure marker

Metabolism of endogenous substrates

- *Calanus* GST similar to GST-2 family in insects
  → conjugation of lipid peroxidative end products (Singh et al., 2001; Agianian et al., 2003)
  → GST induction may also be a marker for lipid peroxidation

Regulation by xenobiotics

- Naphthalene induces GST in a time- and dose-dependent manner (Hansen et al., 2008)

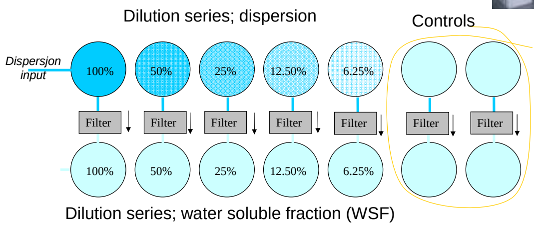


Main objections of study

- Investigate the expression of GST and CYP330A1 following exposure to a realistically weathered North Sea oil
  - Water soluble fractions (WSF) of the oil
  - Dispersed oil (WSF and oil droplets)
- Document relative contribution of oil droplets to these effects
- Explore potential differences in response between lipid rich pre-reproductive females and lipid poor post-reproductive females
- Based on previous experiment with naphthalene, we predicted that:
  - CYP330A1 mRNA levels would be decreased by exposures
  - GST mRNA levels would be increased by the exposures

Experimental principle

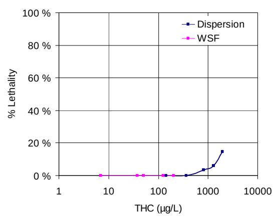
Dispersion made from weathering of a North Sea
75 adult female copepods in each container



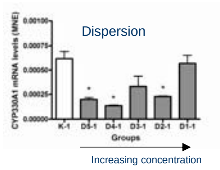
Copepods photographed and sampled individually in RNAlater after 96 hours
→ Pooled groups of 5 specimen in 6 replicates (3 lipid rich and 3 lipid poor)

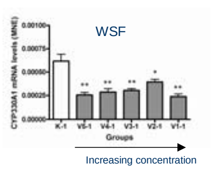
Effects on survival

- Very low lethality in the experiment (<20%)
- Marginally higher lethality in the groups exposed to dispersion than WSF
- THC of dispersions was in the range 0.15 – 2 mg/L (LC50 for *C. finmarchichus* exposed to water soluble fractions (WSF´s) of tested Norwegian oils with different initial loading and weathering degrees is in the range 0.9 -6.3 mg/L)
- Sub-lethal and environmentally realistic exposure



CYP330A1 mRNA levels



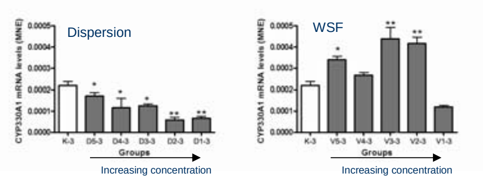


Pre-reproductive females (Fem1)

- Decreased transcription in all groups exposed to WSF and some for dispersed oil
  → Indicating effects on lipid metabolism
- Not concentration-dependent
- Oil droplets do not seem to contribute to this effect compared to WSF alone

Pre-reproductive lipid rich female

CYP330A1 mRNA levels



Post-reproductive females (Fem3)

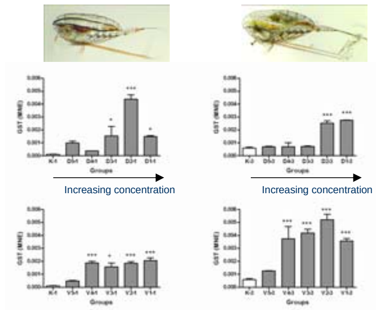
- Decreased transcription observed only when exposed to dispersed oil (concentration-dependent)
- Increased transcription when exposed to WSF alone

Post-reproductive lipid-poor female

GST mRNA levels

Dispersion

WSF



Oil droplets bioavailability

- Oil droplet size range 4-14 µm (average 8 µm)
- Algae *Isochrysis galbana* (5-6 µm)

Low dispersion concentration

- At low concentrations (THC = 0.15 mg/L) of oil dispersion, oil droplets and algae are visible inside the gut

High dispersion concentration

- At high concentrations (THC = 2.0 mg/L), oil droplets appear to stick to the surface of copepods
- Feeding stops

Conclusions

- Acute toxicity is dependent on lipid content and developmental stage of the copepods: **Survival of the fattest**!
- Lipid-rich and lipid-poor female copepods respond differently (CYP330A1 and GST gene expression)
- Exposure to naphthalene, WSF and dispersed oil induce transcription of GST, hence indicating lipid peroxidation
- Exposure to WSF, dispersed oil and high concentrations of naphthalene significantly decrease transcription of CYP330A1, hence indicating effects on lipid metabolism and possibly ecdysteroid synthesis
- Oil droplets are within the size of copepod food, and are therefore available for consumption. However, they do not seem to contribute to the observed effects on gene expression, but they have effects on feeding
- These mechanisms may represent mechanistic modes of toxicity for the established effects of oil exposure on copepod reproduction

Acknowledgements

- Co-authors: Dag Altin, Trond Nordtug and Anders J. Olsen
- Siv-Hege Vang, Kristine Mordal Hessen and Ingeborg Rønning
- Ulrika Dahl and Magnus Breitholtz (University of Stockholm)
- Project was financed by SINTEF Materials and Chemistry, StatoilHydro, ENI Norge and the Norwegian Research Council

Thank you for your attention!