SINTEF
www.sintef.no
www.copepoda.info

UNDERSTANDING MODES OF OIL TOXICITY IN THE COPEPOD *CALANUS FINMARCHICUS*

NTNU
BioTrix

BJØRN HENRIK HANSEN[a,*], DAG ALTIN[b], TROND NORDTUG[a] & ANDERS J. OLSEN[c]

[a]SINTEF – Materials & Chemistry, Marine Environmental Technology, Trondheim, Norway, [b]BioTrix, Trondheim, Norway, [c]Department of Biology, NTNU, Trondheim, Norway, *Corresp.author bjorn.h.hansen@sintef.no

WHY FOCUS ON *CALANUS FINMARCHICUS*?

- Annual production 300 mill. tons
- Constitutes 90% of zooplankton standing stock
- Important food for commercial fish species
- High levels of lipids (up to 50%)

Marine ecological key species in the northern Atlantic Ocean and the Barents Sea

WHAT DO WE KNOW?

Copepods are **sensitive** to environmental stressors, like oil compounds. Oil exposure causes effects on **reproduction**, **development** and **growth**

WHAT DO WE WANT TO FIGURE OUT?

Which **molecular mechanisms** are involved in the effects on reproduction and development?

Can we use molecular markers in test systems to **predict toxicity** of oil compounds?

WHAT HAVE WE DONE??

Established a multi-generation continuous **culture** of *C. finmarchicus*

Sequenced genes expressed during exposure to oil (subtractive suppression hybridization)

Related gene expression to **physiologically important mechanisms** (development and reproduction)

Studied the **impact on oil** on gene expression, hence potential impact on reproduction and development

Copepodite V: Building up lipid reservoir before moult

MOULT

Pre-reproductive female (Fem1): Developing and maturing eggs at the expense of the lipid reservoir.

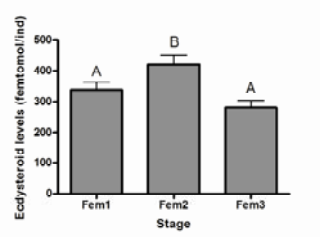
Reproductive female (Fem2): Matured eggs ready to be released. Small lipid reservoir.

Post-reproductive female (Fem3): Released all eggs and no more lipid reserves…end of the line!

ECDYSTEROIDOGENESIS

A total of 6 nauplii and 5 copepodite stages before becoming adults (moulting).

Ecdysteroids initiates the moulting process, a process that takes place when the lipid content is optimal

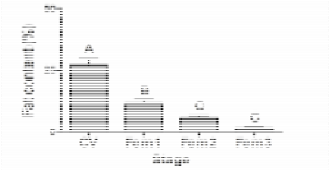


Ecdysteroid levels are high during egg maturation.

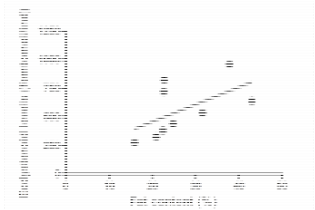
Ecdysteroids are also involved in egg maturation, hence disturbances in ecdysteroidogenesis may cause **delayed development and altered reproduction**

LIPID TURNOVER

Buildup of substantial lipid reservoir is a prerequisite for reproductive success.



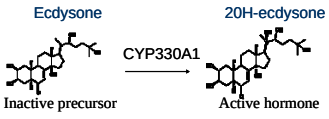
Lipids are utilized during egg production

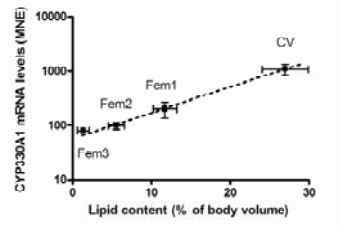


Relationship between ecdysteroids and lipid content in CV. Hence, disturbance in lipid consumption or lipid peroxidation may cause **reduced reproductive success**

CYTOCHROME P450 330A1

CYP330A1 gene expression is induced by the inactive precursor ecdysone, but not by the active hormone 20H-ecdysone (Rewitz et al., 2003).





Relationship between CYP330A1 gene expression and lipid storage size in CV and adult females.

GLUTATHIONE S-TRANSFERASE

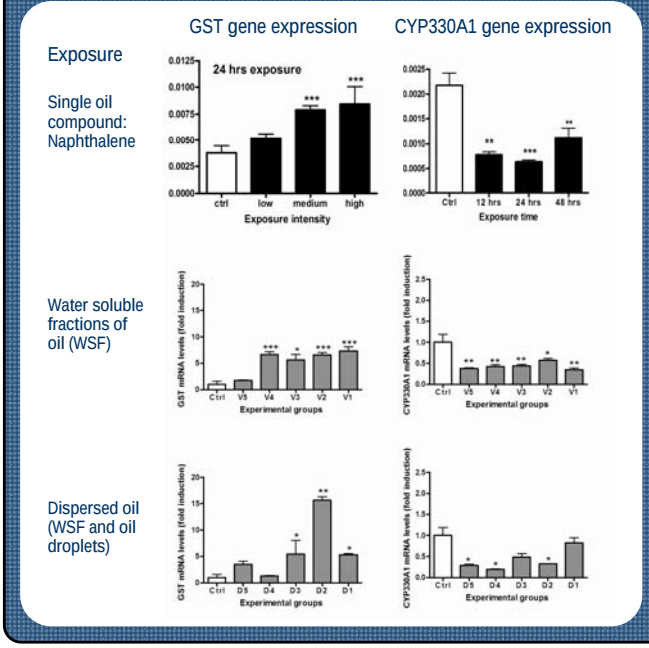
GST is part of a biotransformation system for endogenous and exogenous substrates.

GST has e.g. been found to conjugate reduced glutathione (GSH) onto lipid peroxidative end products (Singh et al., 2001; Agianian et al., 2003).

Marker for lipid peroxidation and altered lipid metabolism.

Oil-induced alterations in CYP330A1 and GST gene expression may represent be a mechanistic link to the well-known negative effects of oil exposure on copepod development and reproduction

EFFECTS OF OIL EXPOSURE ON CYP330A1 AND GST GENE EXPRESSION



CYP330A1:
Altered transcription of CYP330A1 by naphthalene, WSF and dispersed oil indicates effects on ecdysteroid metabolism and lipid turnover

GST:
Induction of GST by naphthalene, WSF and dispersed oil indicates lipid peroxidation, hence effects on lipid turnover.

Oil droplets visualized by fluorescence. Oil droplets (at high concentrations) stick to the surface of the copepods and alter feeding. At lower concentrations droplets are ingested.

Related references:

Hansen, B. H., Altin, D., Hessen, K.M., Dahl, U., Breitholtz, M., Nordtug, T., Olsen, A. J. Expression of ecdysteroids and cytochrome P450 enzymes involved in lipid turnover and reproduction in *Calanus finmarchicus* (Crustacea: Copepoda). Submitted to General and Comparative Endocrinology.

Hansen, B.H.; Nordtug, T.; Altin, D.; Booth, A.; Olsen, A.J., Hessen, K.M. Expression of CYP330A1 and GST lipid-rich and lipid-poor *Calanus finmarchicus* following exposure to oil droplets and water soluble fractions of oil. Manuscript submitted to Journal of Toxicology and Environmental Health.

Hansen, B.H.; Altin, D.; Vang, S.-H.; Nordtug, T.; Olsen, A.J., 2008. Effects of naphthalene on stress gene transcription in *Calanus finmarchicus* (Crustacea; Copepoda). Aquatic Toxicology, 86, 157-165.

Hansen, B.H.; Altin, D.; Nordtug, T.; Olsen, A.J. 2007. Suppression subtractive hybridization library prepared from the copepod *Calanus finmarchicus* (Gunn.) exposed to a sublethal mixture of environmental stressors. Comparative Biochemistry and Physiology.: Part D: Genomics and Proteomics, 2, 250-256.