
 **Expression of ecdysteroids and fat content as a useful tool for understanding development and reproduction in environmental risk assessment of *Calanus finmarchicus*.**

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 **Background**

Crustaceans as test animal

- Invertebrates comprise 95% of all animal organisms on earth
- Crustaceans is the second largest subphylum
- 42.000 different crustacean species


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 **Background**

What do we know?

- Copepods commonly used in Environmental Risk Assessment
- Copepods are sensitive to environmental stressors


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 **Background**

What do we know?

- Basic knowledge about the different test species is limited
- Copepod endocrine system is still a black box
- Dissectional procedures; e.g. removal of organs for physiological understanding, not yet possible in such small organisms


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 **Background**

What do we want to figure out?

- A way of measuring ecdysteroids in individual copepods (1-2 µg dry weight)
- Which molecular mechanisms are involved in the effects of reproduction and growth?

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 **Background**

Why focus on *Calanus finmarchicus*?

- Marine ecological key species in the northern Atlantic Ocean and the Barents Sea
- Annual production 300 mill. tones
- Constitutes 90% of zooplankton standing stock

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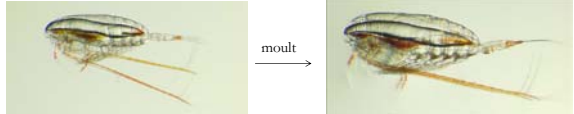
Background
Why focus on *Calanus finmarchicus*?

- High levels of lipids (up to 50%)
- Important food for commercial fish species

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Background
Calanus finmarchicus

- SINTEF (Norway) established a continuous *C. finmarchicus* stock culture

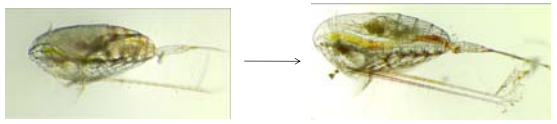


Copepodite CV, building up lipid reservoir before moult

Pre-reproductive female (Fem1), developing and maturing eggs at expense of lipid reservoir

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Background
Calanus finmarchicus




Reproductive female (Fem2), matured eggs ready to be released. Small lipid reservoir

Post-spawning female (Fem3), released all eggs and empty lipid reservoir

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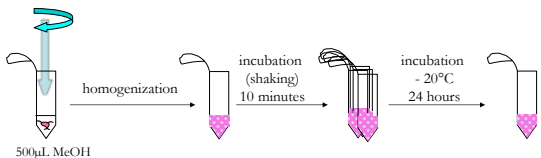
Background
Ecdysteroids

- Total 6 nauplius and 5 copepodite stages before maturing into adult
- Ecdysteroids initiates the moulting process, by the release of inactive ecdysone converting into active 20-hydroxyecdysone



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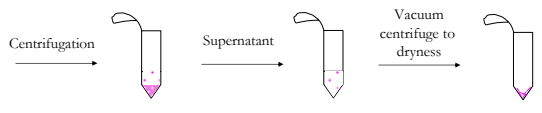
Material and Method
Ecdysteroid content
Enzyme immunoassay



500µL MeOH

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Material and Method
Ecdysteroid content
Enzyme immunoassay



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Material and Method
Ecdysteroid content
Enzyme immunoassay

50µL · replicate¹

50µL · replicate¹

Scanning

- anti-20hydroxyecdysone (20HE) rabbit polyclonal antisera (secondary antibody)
- 20HE horseradish peroxidase (conjugate)

λ_{ex} 340/30 nm
 λ_{em} 420/50 nm

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Material and Method
Ecdysteroid content
Enzyme immunoassay

- IgG, Fc specific (primary antibody)
- blocking buffer
- anti-20HE antibody (secondary antibody)
- sample / standard
- 20HE HRP (enzyme conjugated secondary antibody)

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Material and Method
Ecdysteroid contents
Enzyme immunoassay

Excess washed out

- IgG, Fc specific (primary antibody)
- blocking buffer
- anti-20HE antibody (secondary antibody)
- sample / standard
- 20HE HRP (enzyme conjugated secondary antibody)

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Material and Method
Ecdysteroid contents
Enzyme immunoassay

Fluorescent quantification, inversely proportional to antigen concentration

- IgG, Fc specific (primary antibody)
- blocking buffer
- anti-20HE antibody (secondary antibody)
- sample / standard
- 20HE HRP (enzyme conjugated secondary antibody)

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Material and Method
Biometric methods
Lipid content quantification

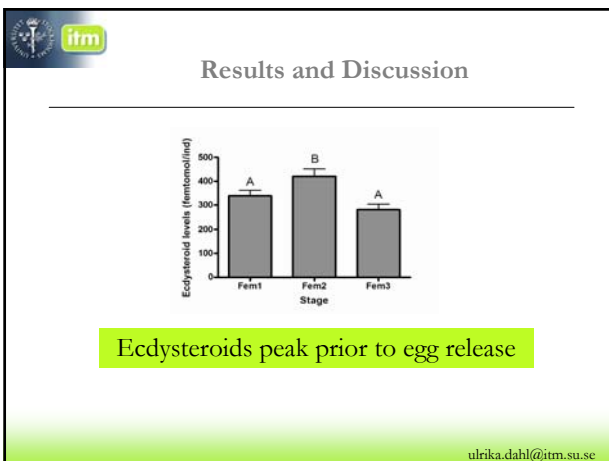
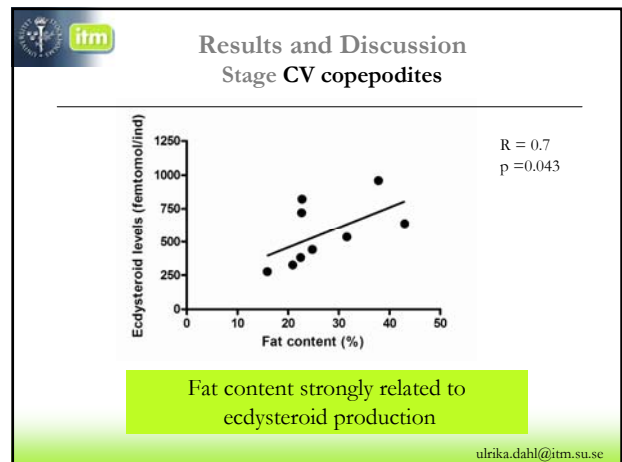
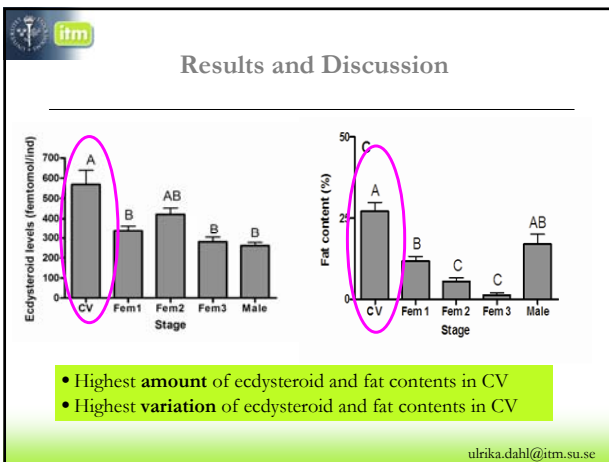
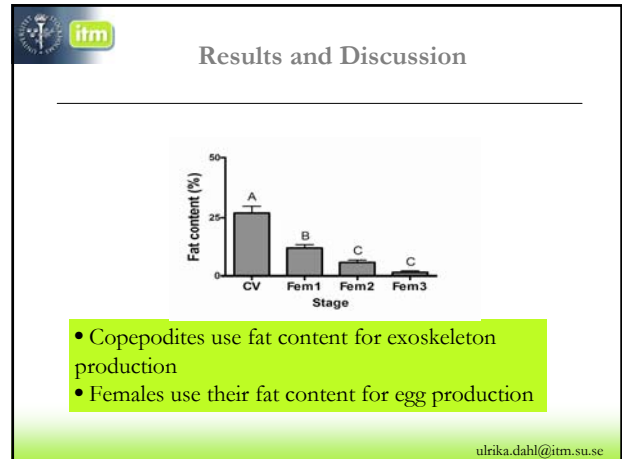
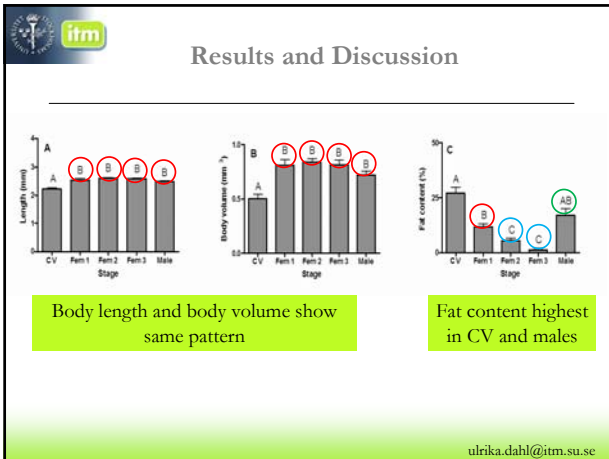
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Material and Method
Results and Discussion

Stage	Ecdysteroid levels (femtomol/ind)
CV	~580 (A)
Fem1	~350 (B)
Fem2	~420 (AB)
Fem3	~300 (B)
Male	~280 (B)

Ecdysteroid levels highest in CV and females with eggsac

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Conclusion

- The enzyme immunoassay of individual copepods is a useful tool for investigations of the endocrine system of copepods with diminutive biomass
- The present results clarify an important link in understanding some of the biochemical features in copepods

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Thank you for your attention

Further reading:

- 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*.
- Dahl U., Breitholtz M., (in press). Integrating individual ecdysteroid content and growth-related stressor endpoints to assess toxicity in a benthic harpacticoid copepod. *Aquatic Toxicology*.