FACULTY OF BIOSCIENCE ENGINEERING

Harpacticoid copepods in a DEB framework: Investigating pharmaceutical effects on *Nitocra spinipes*

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Introduction

- **Copepods** are an ideal test system in ecotoxicology studies:
  - Largest animal biomass on earth (estimate)
  - Small size
  - Easy lab culture and handling
- **Dynamic Energy Budget (DEB) theory** can help to identify a stressor's mode of action (MoA) on energy allocation
- The copepod life cycle **deviates from standard DEB**:
  - Complete metamorphosis after 6th molt
  - Abrupt stop in growth at adult stage

➔ Copepods need further investigation for use in a DEB framework

Materials & Methods

Life cycle experiments

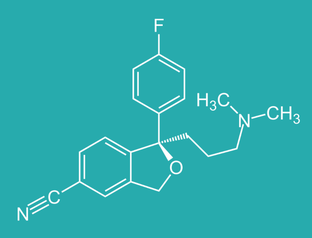
- Experimental setups were based on the OECD guidance document [1] for harpacticoid copepod life cycle testing

Test species: *Nitocra spinipes*

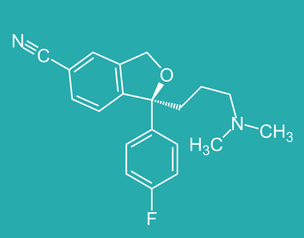
- Brackish water species
- Worldwide distribution
- Test species since 70s

Test compound: Citalopram

- Selective serotonin re-uptake inhibitor (antidepressant)

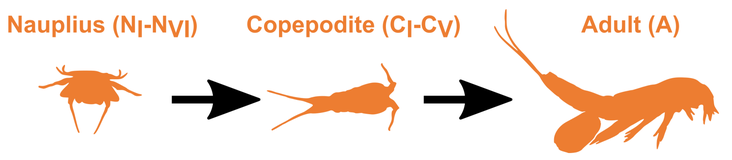


(*R*)-citalopram

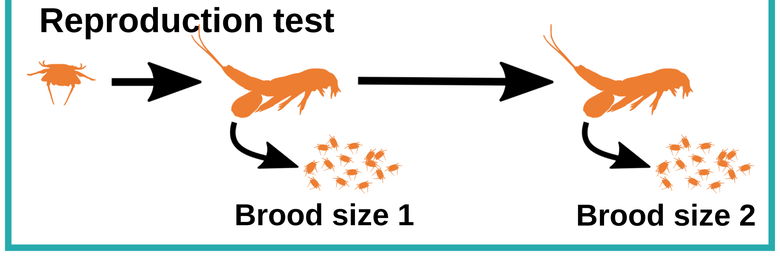


(*S*)-citalopram

Development test



Reproduction test



Model calibration

- **DEBKiss** (*'Keep it simple, stupid')* [2] was calibrated on development time and reproduction data at different food levels [3]
- Length per life stage at control conditions was measured to **convert development time to body length over time** (Asm. 1)
- The **shape correction coefficient** (vol. body length × real body length^{-1}) was implemented as a **function of size** to account for changes in morphology
- **Growth is terminated at the adult stage**
- Further modifications include Asm. 2 and 3

❑ **Assumption 1**: The body length at a certain life stage is independent of food and chemical stress

❑ **Assumption 2**: Adults invest all energy not used for somatic maintenance into reproduction

❑ **Assumption 3**: The specific assimilation rate is shifted up at one of the early copepodite stages

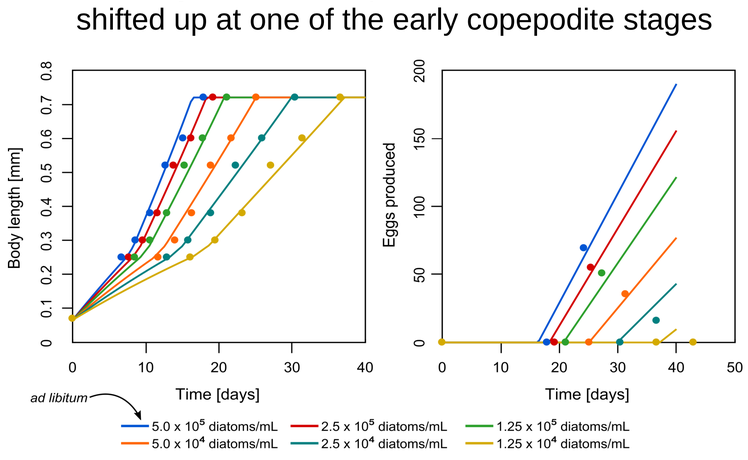


Fig. 1: DEBKiss model calibrated on development and reproduction data of *N. spinipes* at six food levels.

Results & Discussion

Development and reproduction test

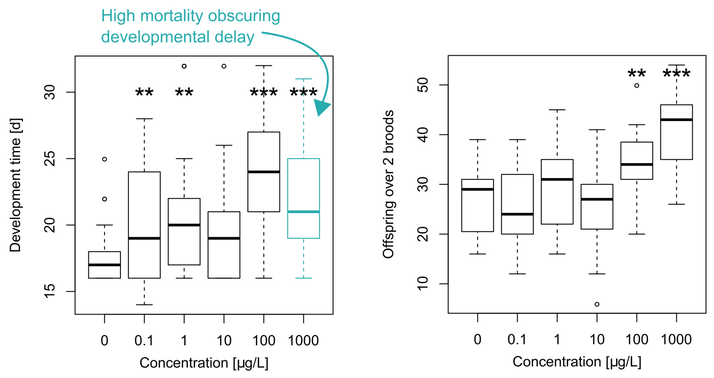


Fig. 2: Development time from nauplius to adult (left panel) and offspring over two broods per female (right panel). Data were analyzed in one-way ANOVA with a one-sided Dunnett's test (***p < 0.001 **p < 0.01 *p < 0.05).

- Slight **developmental delay** at 100 ng/L and stronger delay at and above 100 µg/L
- **Brood stimulation** at and above 100 µg/L

Identification of DEB-MoA

- Observed effects at 100 and 1000 µg/L were simulated in the DEBKiss model by means of the **stress factor *s***
- A **slope parameter *a*** was introduced to the stress function to accurately cover the concentration dependent **magnitude of effects**

$$s = \frac{1}{c_T} \times \max(0, c_V - c_0)^{\,a}$$

s = Stress factor on MoA parameter
c_0 = No-effect concentration
c_T = Tolerance concentration
a = Slope parameter
c_V = Scaled internal concentration

- Observed effects could be explained by the presence of **two individual MoAs**:

(a) Increase in growth costs

(b) Decrease in reproduction costs

- The stress function could be calibrated with just one set of parameters to describe both effects (instead of using separate fits for each MoA)

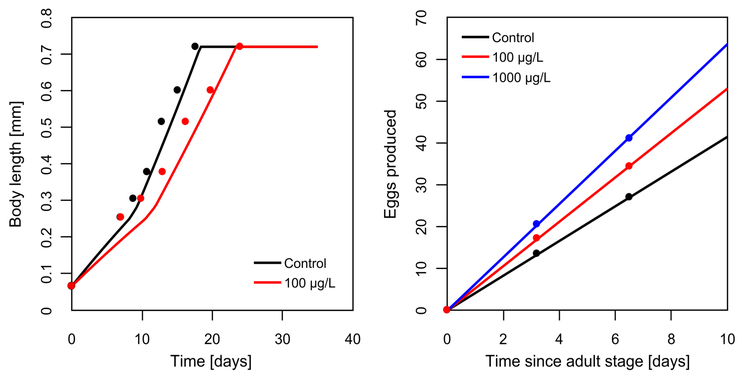


Fig. 3: DEBKiss model fits for observed effects.

Conclusions

- The *N. spinipes* life cycle could be captured well by just slight modifications of the DEBKiss model structure
- Effects of citalopram on development (inhibition) and reproduction (stimulation) of *N. spinipes* were explained by MoAs on energetic costs for growth and reproduction

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References

[1] OECD. New Guidance Document on Harpacticoid Copepod Development and Reproduction Test with *Amphiascus*. Environmental Health and Safety Publications. Series on Testing and Assessment No. 201. Env/Jm/Mono(2014)17. Paris. 2014.
[2] Jager T, Martin BT, Zimmer EI. DEBkiss or the Quest for the Simplest Generic Model of Animal Life History. J Theor Biol. 2013;328: 9-18.
[3] Koch J, Bui TT, Lundström Belleza E, Brinkmann M, Hollert H, Breitholtz M. Temperature and Food Quantity Effects on the Harpacticoid Copepod *Nitocra spinipes*: Combining *in Vivo* Bioassays with Population Modeling. PLOS ONE. 2017;12(3): e0174384.

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