Isabella Buttino

Effects of diatom aldehydes on copepod reproduction: use of giant liposomes encapsulated with decadienal

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and

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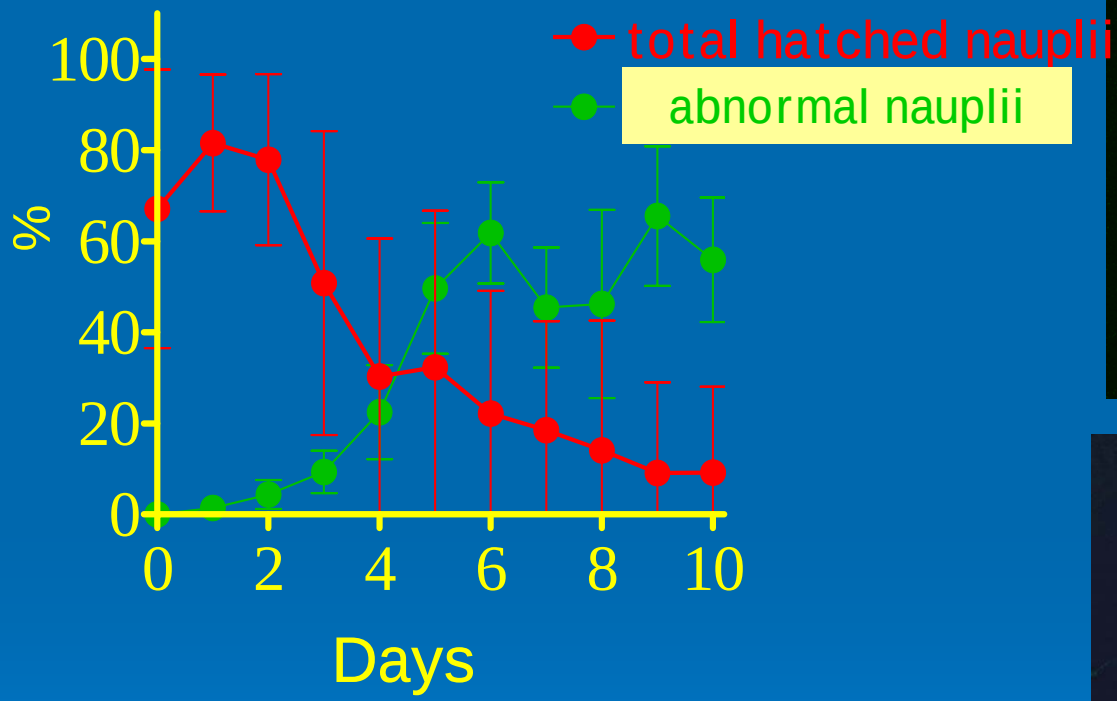
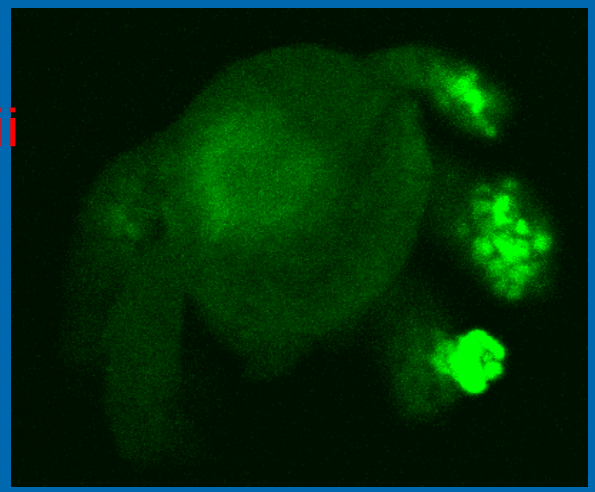
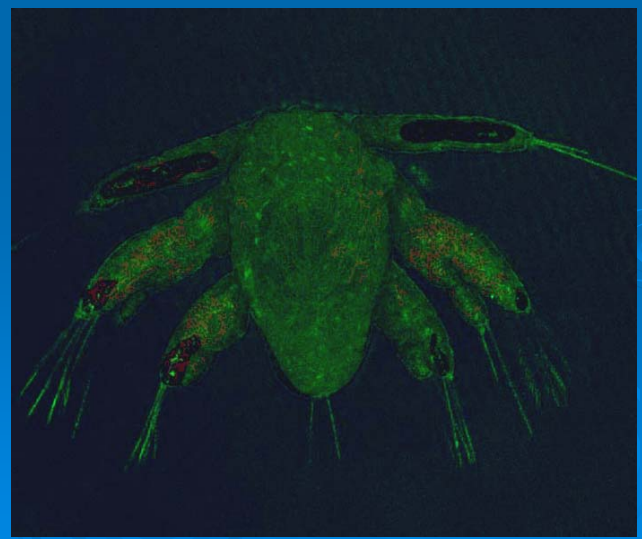
Feeding experiments: *Calanus helgolandicus* fed *Skeletonema costatum* (7.3 x10^4 cells ml^{-1})



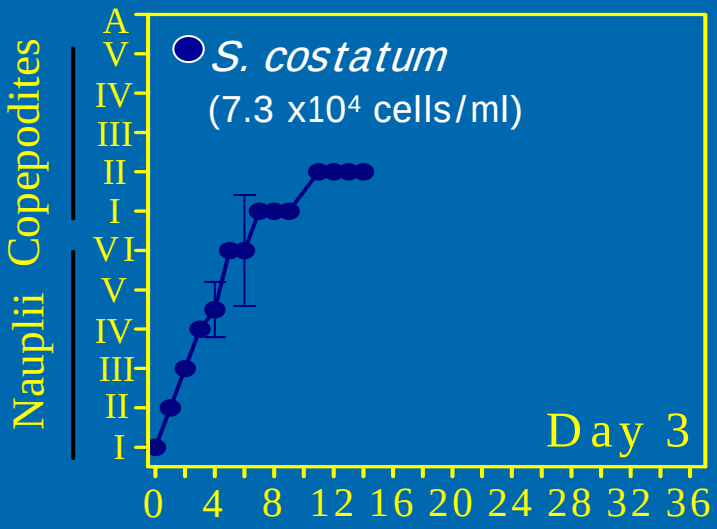
Ianora et al. Nature 2004

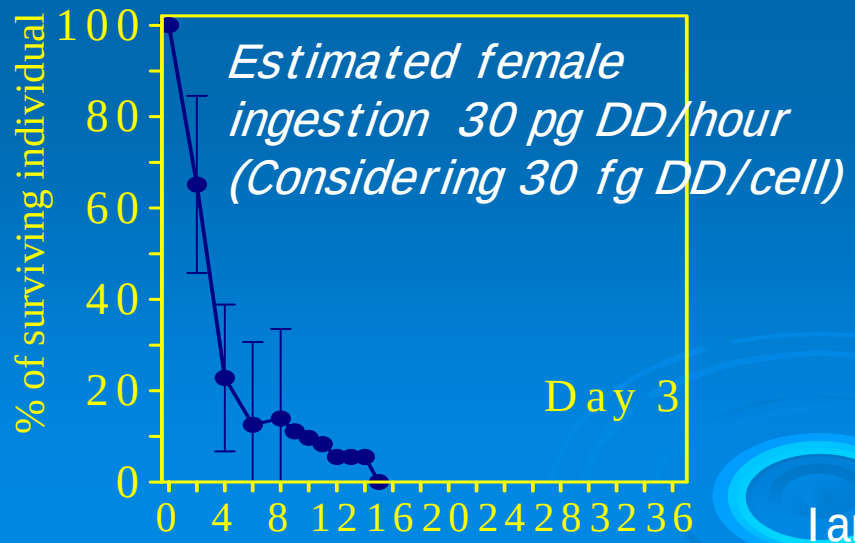




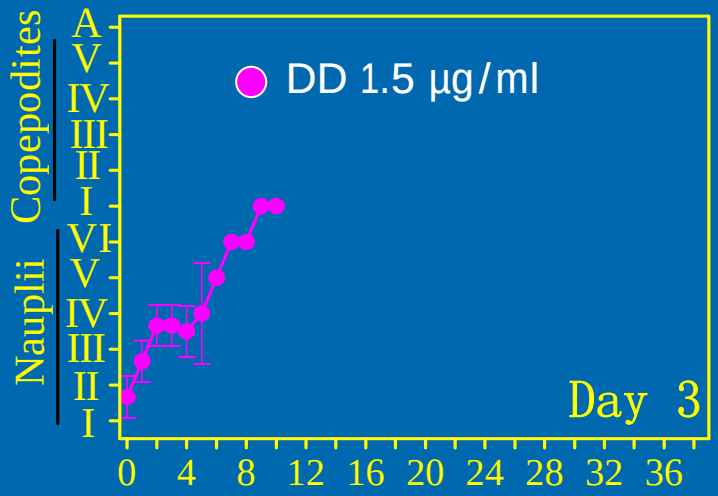
Effects of maternal and neonate diet: Feeding+incubation exp.

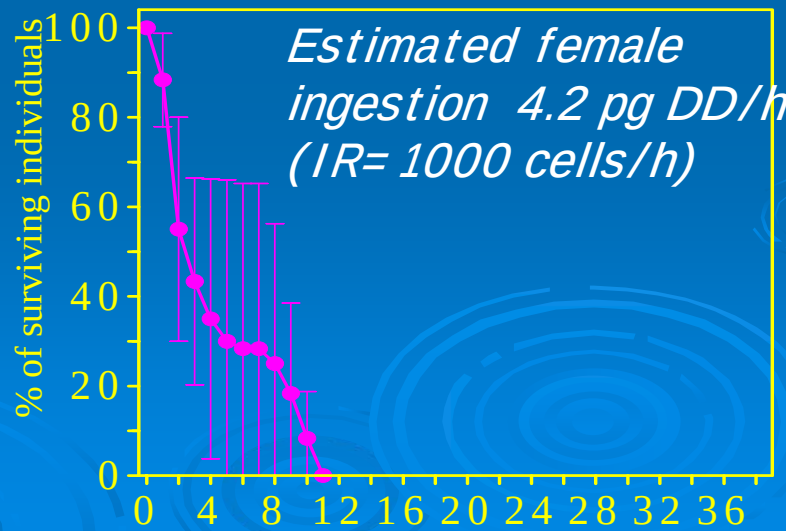
Effects of SKE diet on development rates and % of neonate survivorship





Effects of the aldehyde decadienal (DD + PRO) on development and % survivorship





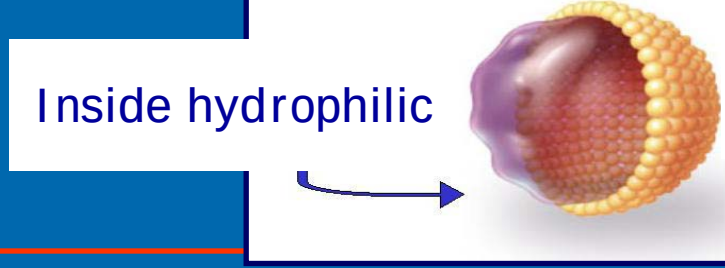
Ianora et al., Nature 2004 (modified)

Aim:

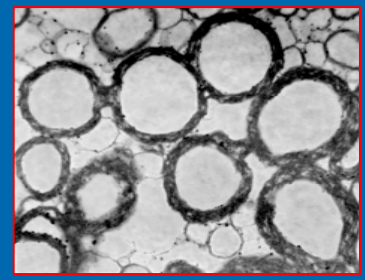
- To establish a precise relationship between decadienal uptake and reproductive failure in copepods
- we prepared inert carriers, LIPOSOMES, encapsulated with a known concentration of decadienal,
- able to deliver diatom-derived aldehyde after copepod ingestion.

Liposomes:

Phosphatidylcholin/Cholesterol
(2:1 mol/mol)



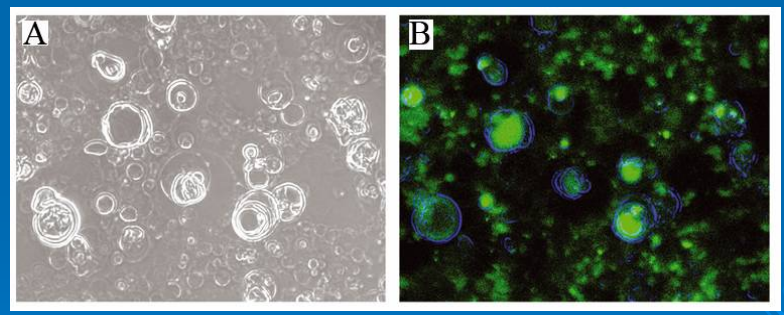
- prepared in the same size range of copepod food (about 7 µm)
- biodegradable and not toxic
- Stable in time (up to 15 days)



- Blank liposomes

Hand-shaking and hydration

- FITC-dextran encapsulating-liposomes



- ^{3}H-cholesterol labelled liposomes
- trans-trans 2,4 decadienal encapsulating liposomes

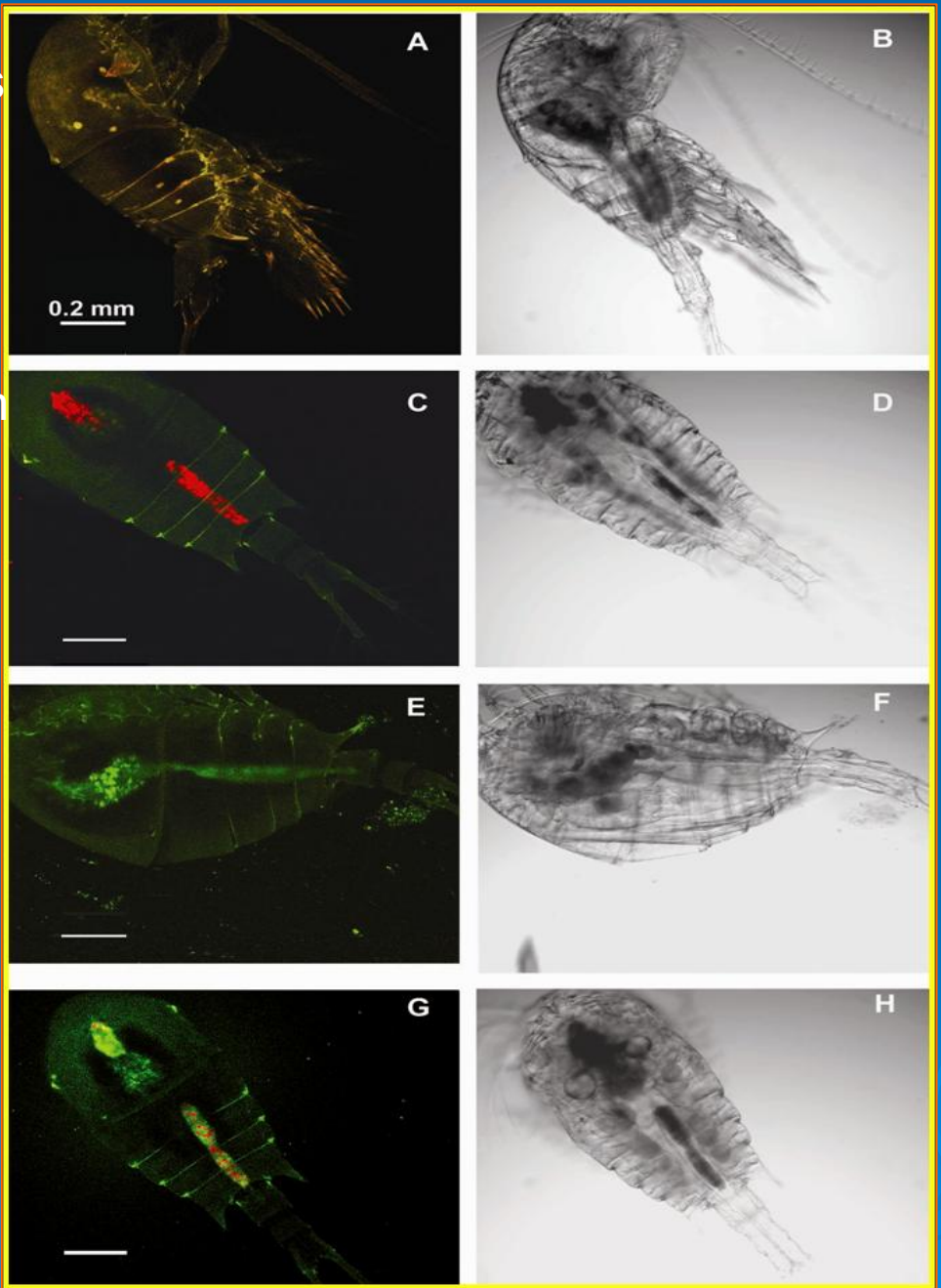
Feeding experiments with FITC-loaded liposomes

Starved copepods

P. minimum for 48h

FITC–liposomes for 48h

P. minimum + FITC–liposomes for 24h



Buttino et al. JEB 2006

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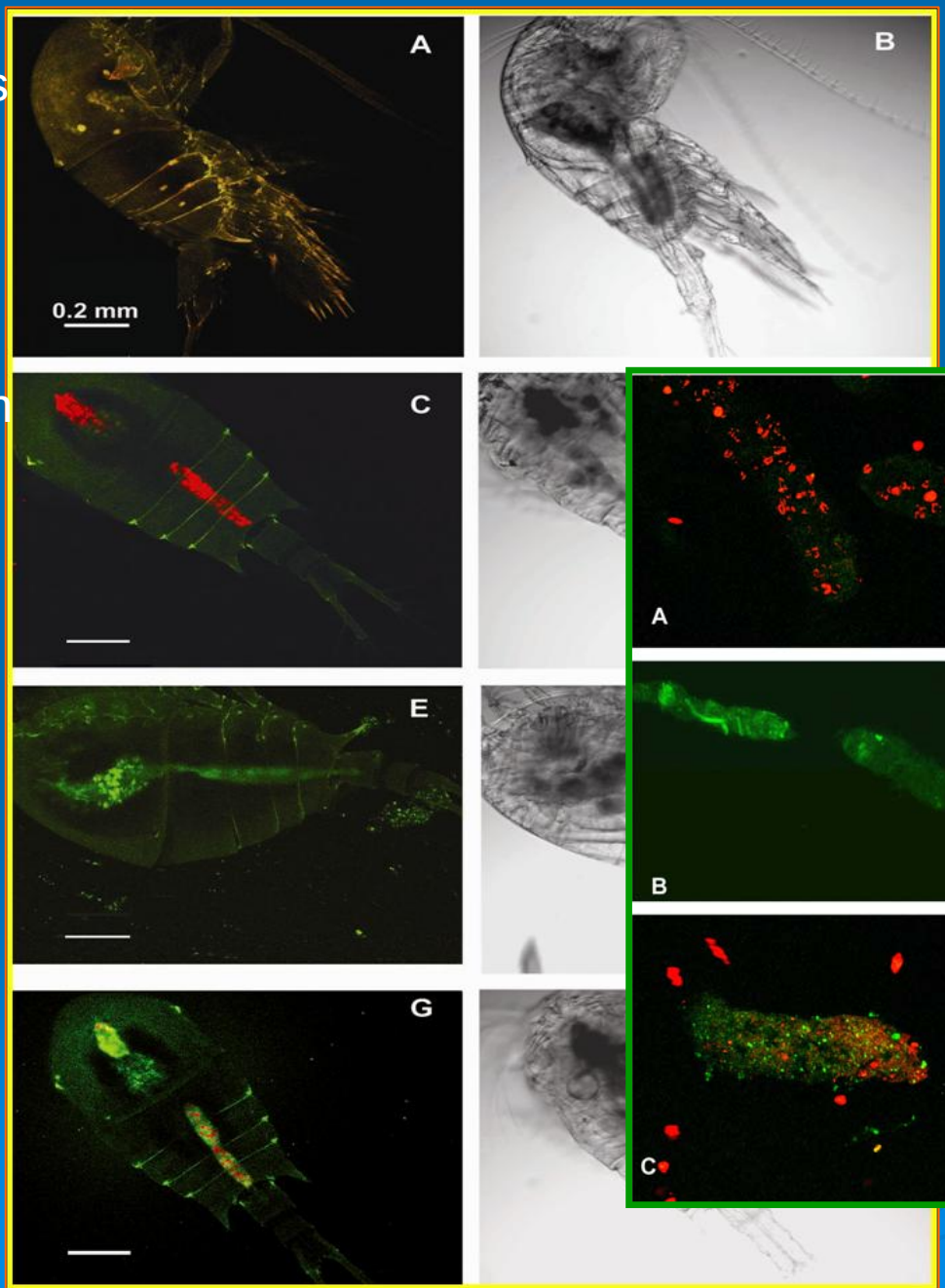
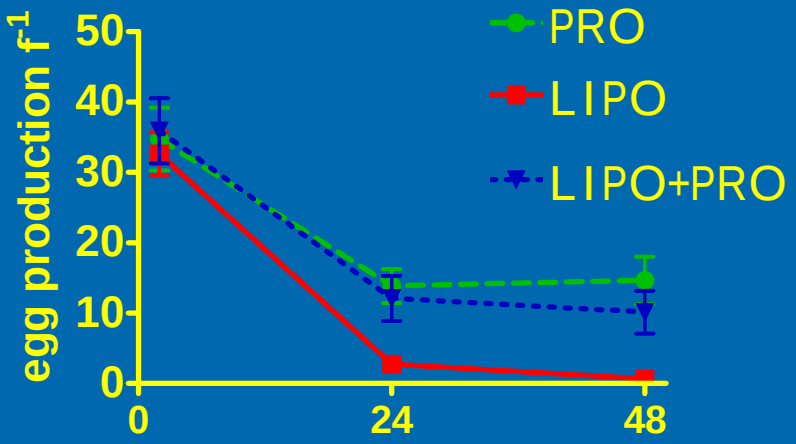
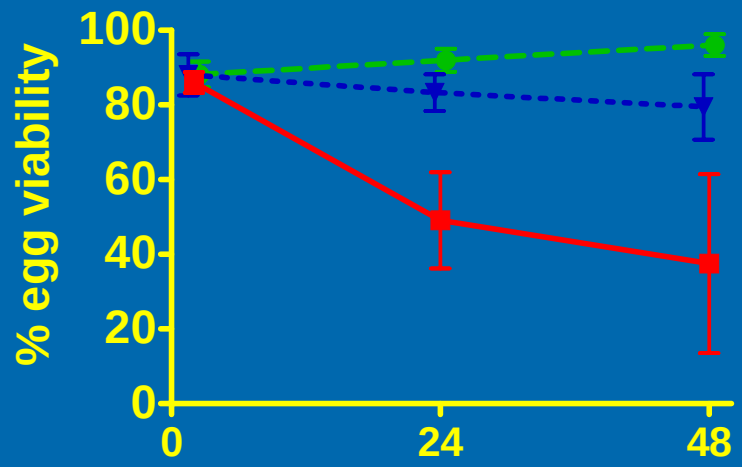
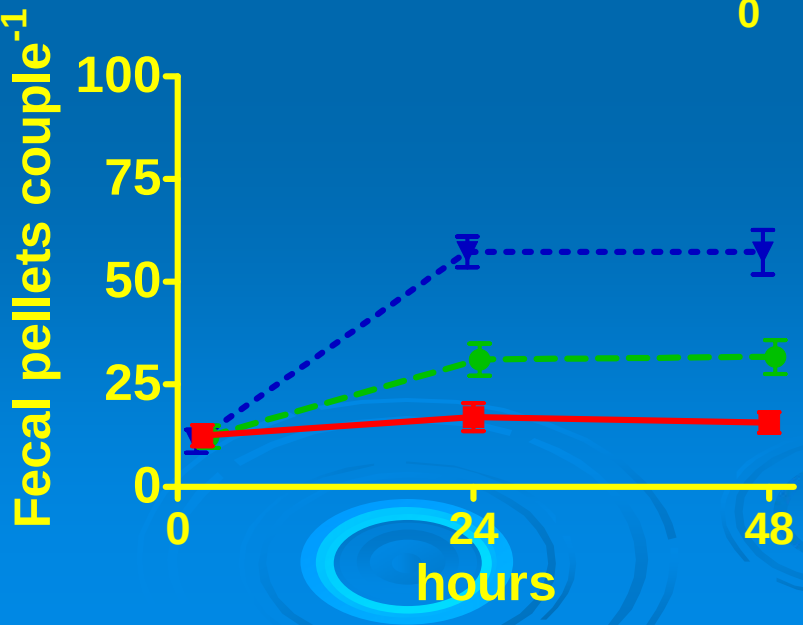


Buttino et al. JEB 2006

Effect of liposome diet on the reproductive physiology of *T. stylifera*



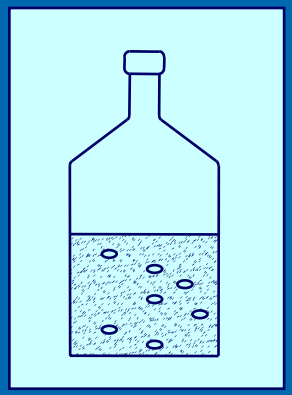




Lipid conc. = 40 µg/ml
(10^5 liposomes/ml)

Buttino et al., JEB 2006

Liposome filtration rate

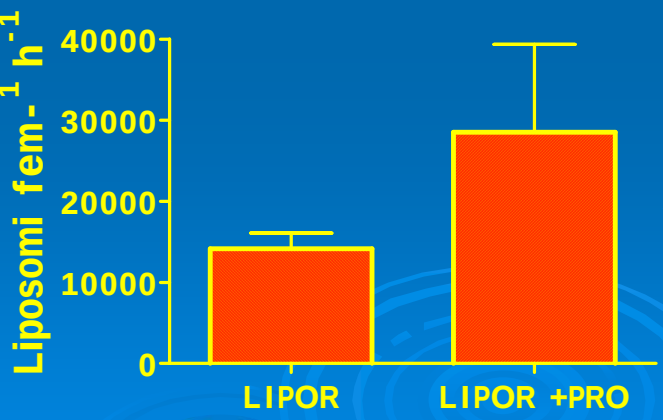


Scintillation counter

Copepods fed 30'
^{3}H-liposomes with and without *P. minimum*

Ingestion rate 0.14 ml/hour

Food type	% ingested liposomes
^{3}H-Liposomes + PRO	16
^{3}H-Liposomes	7.6

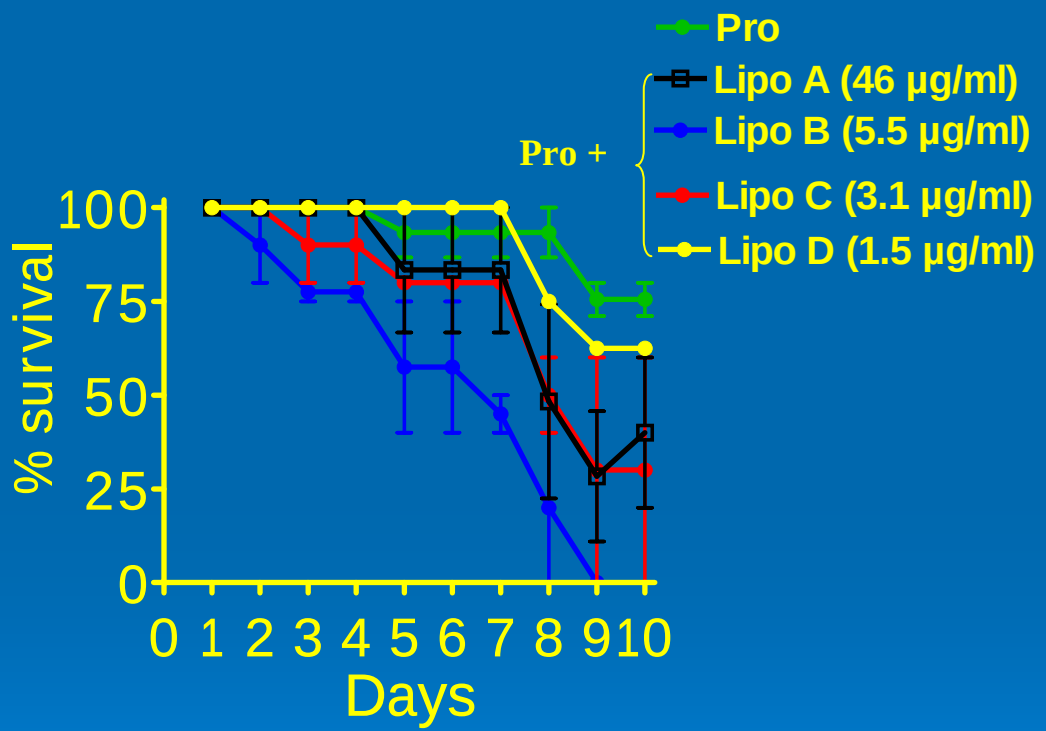


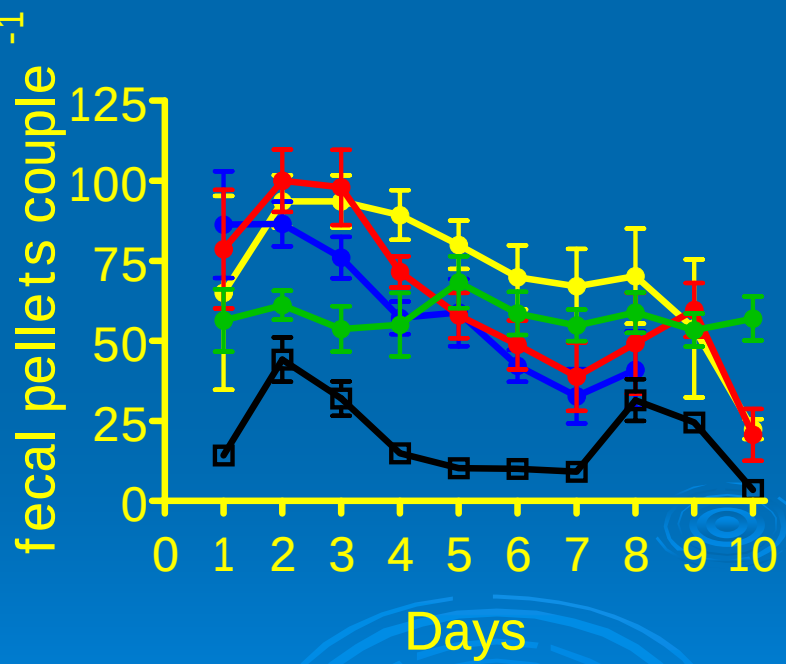
Buttino et al. JEB 2006

Conclusions I

- Liposomes were actively ingested by *T. stylifera* when mixed with the algal food.
- The very low egg production reported when administered alone, suggests that liposomes *per se* did not add any nutritive value to the diet, making them a good candidate as inert carriers to study copepod physiology in ecotoxicological experiments or nutritional requirements studies.

Long-term effects of liposome ingestion at different lipid concentrations





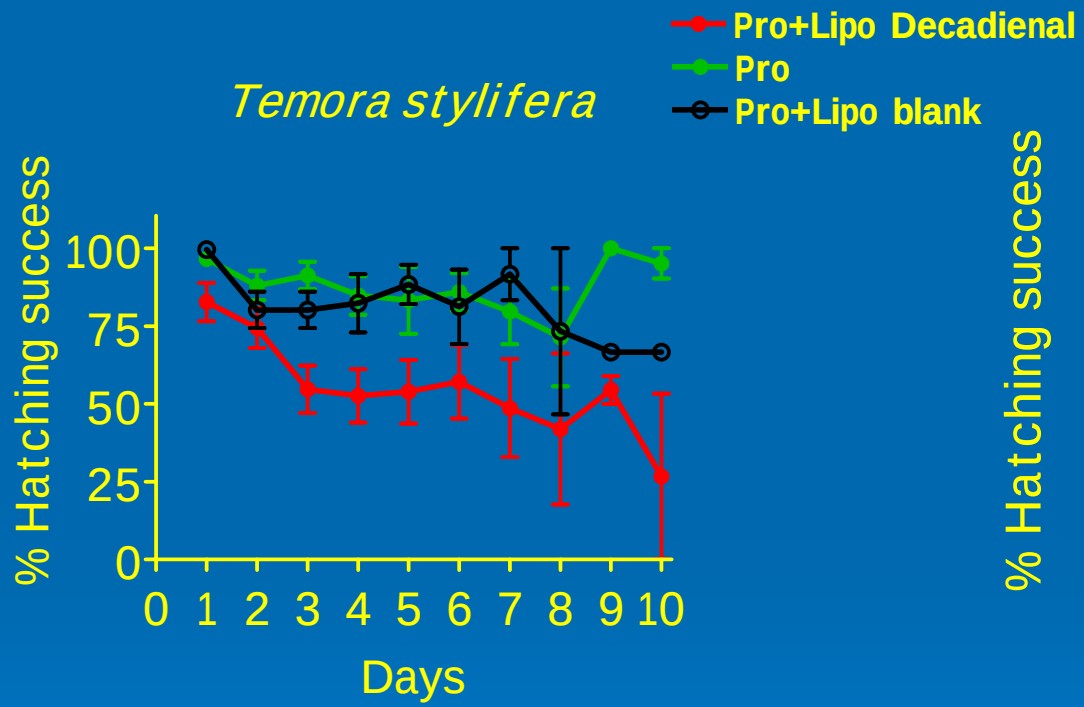
Buttino et al., in prep.

Effects of decadienal-encapsulating liposomes on copepod egg viability

Final concentration of DD in liposomes
3 ± 0.23 ng/ml

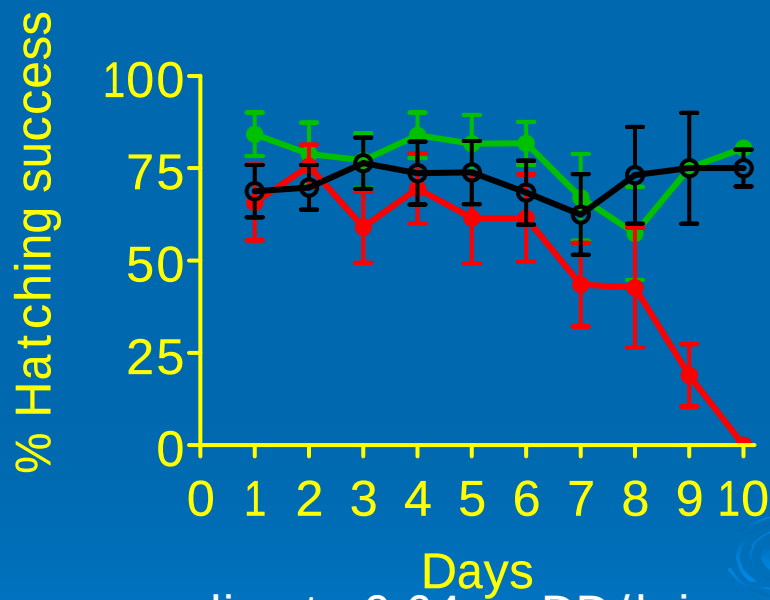
Final concentration of DD in liposomes
4.6 ± 1.2 ng/ml

Temora stylifera



Corresponding to 0.42 ng DD/h ingested

Calanus helgolandicus



Corresponding to 0.64 ng DD/h ingested

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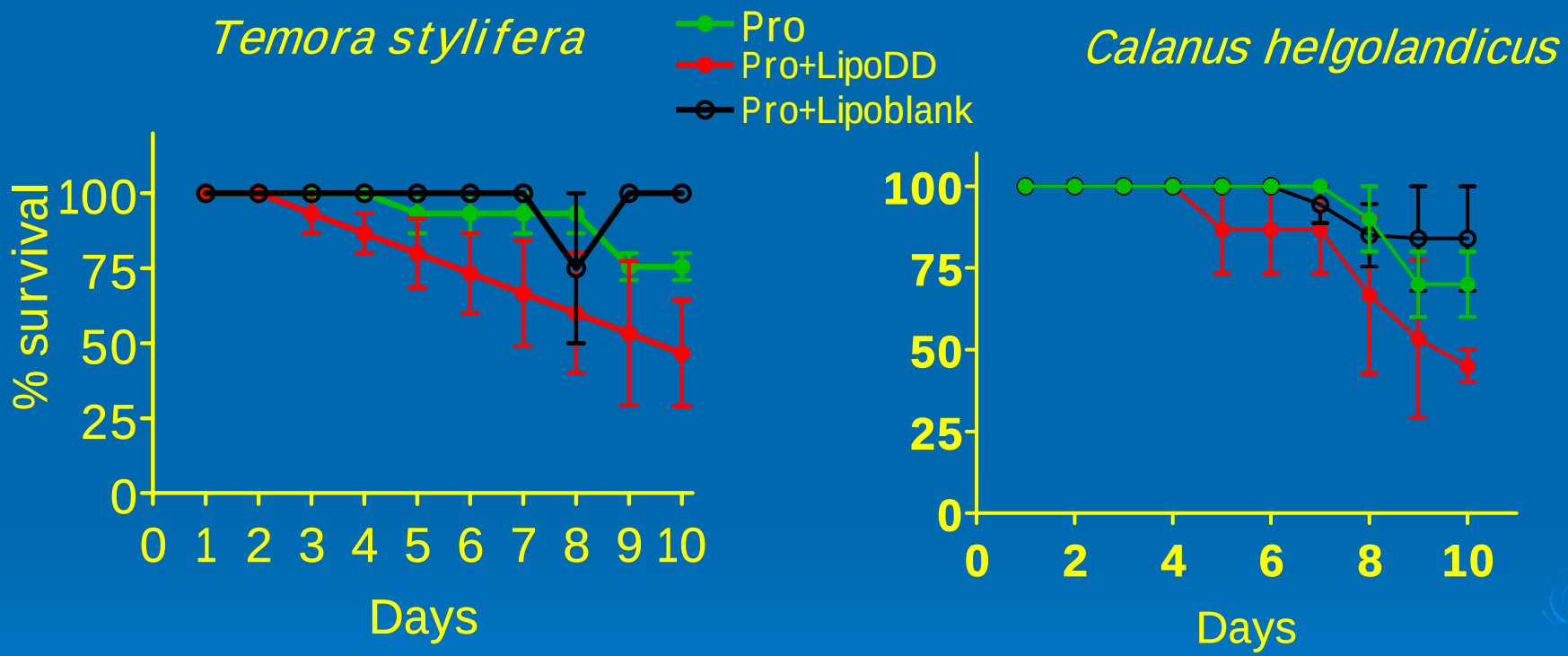
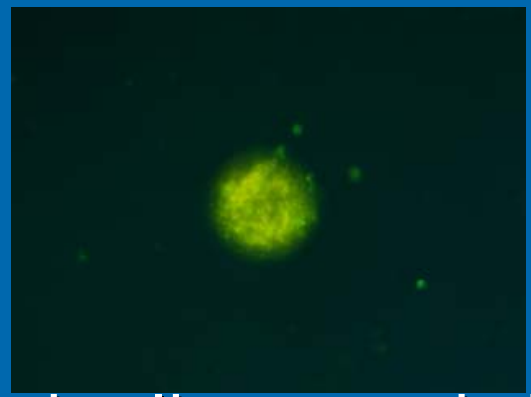
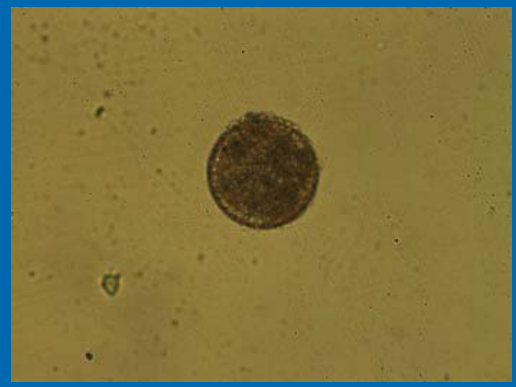
Effects of decadienal-encapsulating liposomes on female survival



Buttino et al., in prep.

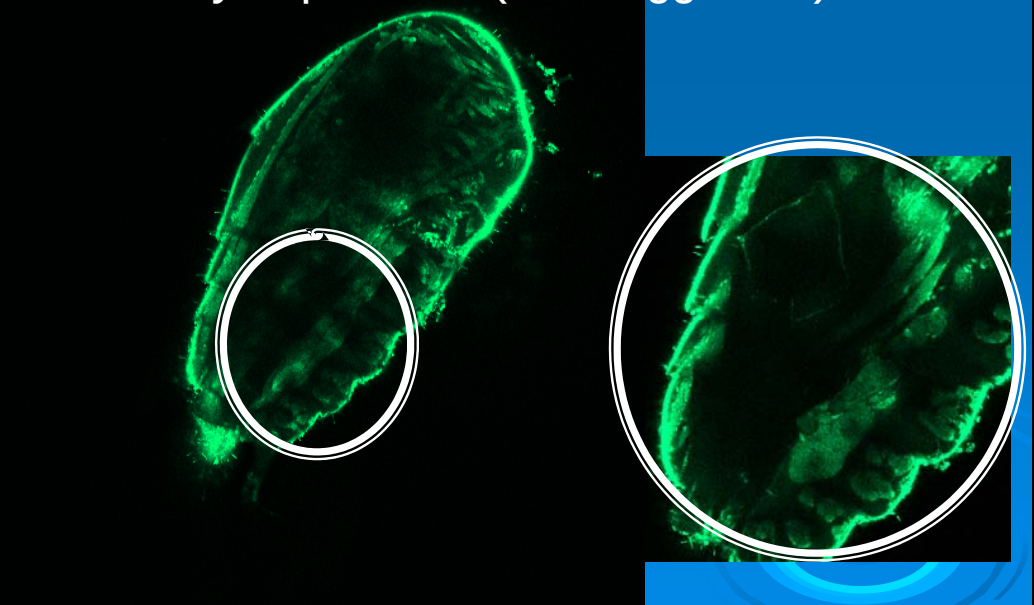
- Decadienal–encapsulating liposomes induce apoptosis in copepod embryos as already observed in feeding and in incubation experiments



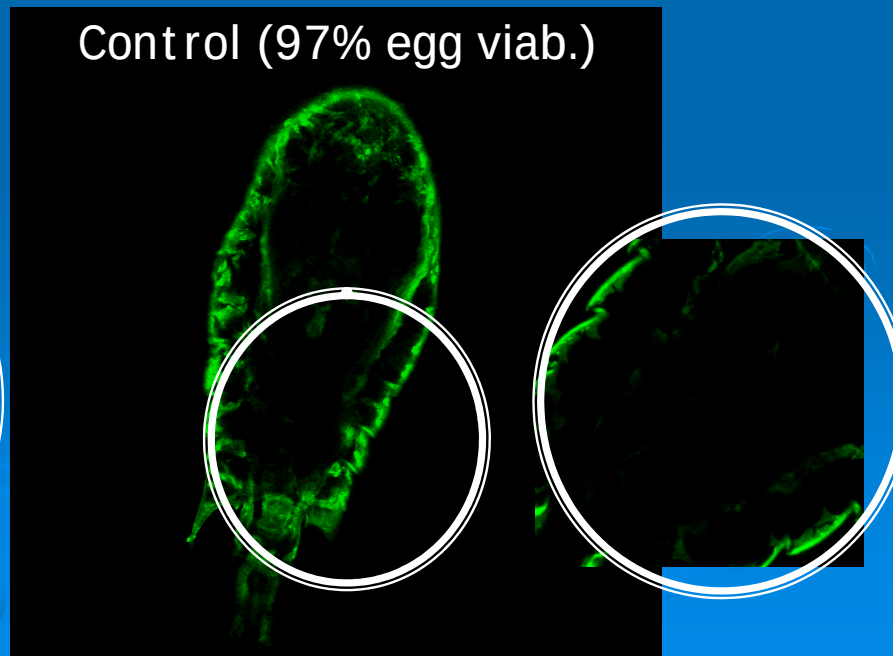


- Do decadienal-encapsulating liposomes induce apoptosis in females?

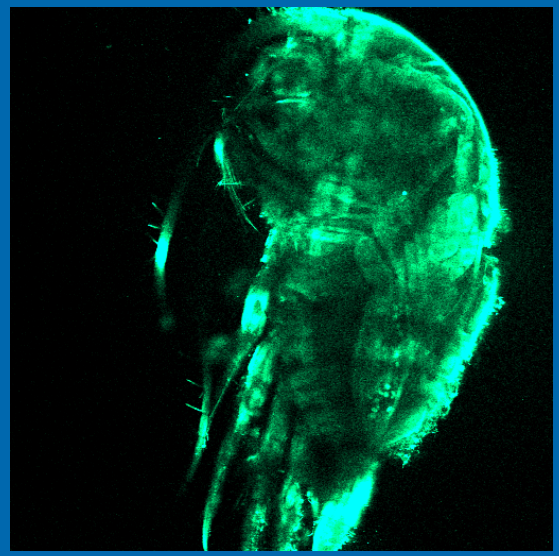
Fed 6 days lipo-deca (33% egg viab.)

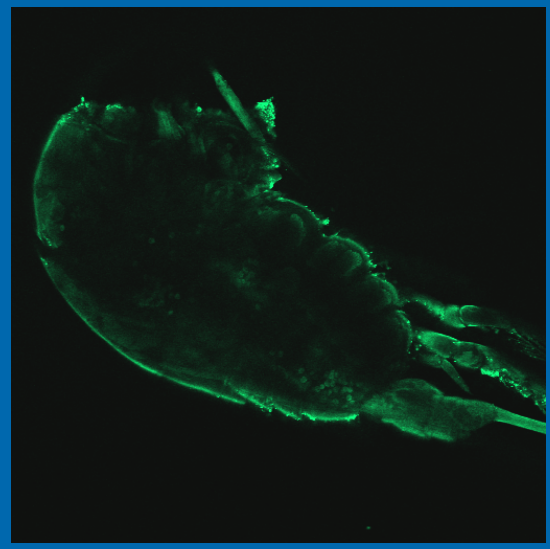


Control (97% egg viab.)

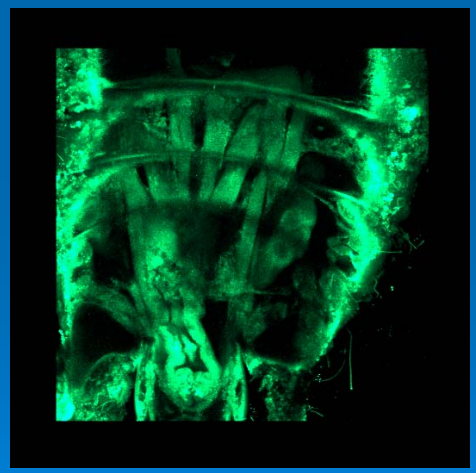


Total body apoptosis





Control female
egg viability 93%



Female fed 10 days
egg viability 7%

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Conclusions I I

- Decadienal-encapsulating liposomes are the only tool allowing to test the effect of a precise concentration of the diatom-derived molecules on the reproductive physiology of copepods.

(diatom-aldehyde production changes with the life cycle of the algae, aldehyde production re-initiated when it is removed by the medium, so it is very difficult to determine the precise quantity of aldehydes per cell).

- This is the first study that rigorously provides evidence that ingestion of DD induces egg mortality in copepods
- The combined effects of different aldehydes (synergistic or antagonistic) can be tested on copepods, modulating their concentrations to better understand phytoplankton-herbivore interactions at sea.
- Threshold aldehyde concentration (known as No Observed Adverse Effect Level) could reveal different sensitivity among copepod species to diatom-aldehyde compounds, helping to understand the ecological role of these metabolites on copepod population.

Thanks to all colleagues

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