

An inter-order comparison of copepod fatty acid composition and biosynthesis in response to a long-chain PUFA-deficient diet along a temperature gradient

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Copepods are a key trophic link in marine food web dynamics, connecting primary producers to higher trophic levels. Recent warming conditions have reduced the concentrations of essential fatty acids (EFAs) in phytoplankton, and it remains unknown if copepods have the adaptive capacity to cope. Calanoid species, *Temora longicornis*, and harpacticoid, *Platychelipus littoralis*, from the North Sea and Westerschelde mudflat respectively, were chosen to characterize the fatty acid (FA) biosynthesis response to a polyunsaturated FA (PUFA) deficient diet (*Dunaliella tertiolecta*) along a temperature gradient. Copepods were fed ¹³C-labelled *D. tertiolecta* to quantify assimilation and *de novo* FA production at 11, 14, 17, 20, and 23 °C. FA composition and ¹³C isotope incorporation were determined using gas chromatography mass spectrometry (GC-MS) and GC-combustion carbon isotope ratio-MS (GC-C-IRMS) respectively.

To compensate for poor quality food organisms can increase the carbon incorporation efficiency (1). Through the use of labelled food we are able to discern this effect on the assimilation and thereby what is retained by the individuals. *T. longicornis* has higher overall rates of assimilation, and maintains this across all temperatures, likely to compensate for the high metabolic costs. This indicates that either this process is independent of temperature in the calanoid, or the experimental stress was heightened enough at 11 °C to induce maximum ingestion rates, to compensate for the stressors. In comparison, *P. littoralis* has relatively low assimilation, only increasing at the highest temperature treatment (23 °C). This in conjunction with the retention of relative field EFA concentrations may indicate that *P. littoralis* does not need to increase assimilation to meet their metabolic demands until the extreme of 23 °C. Standardized per day, carbon assimilation in *P. littoralis* ranges from 0.075 to 0.214% day⁻¹ at 11 and 23 °C respectively, whereas in *T. longicornis* it is 2.014% day⁻¹ on average. Interestingly, the deviation of *T. longicornis* ¹³C assimilation per total lipid increased with temperature, this heightened variability between replicates is recognized as a biochemical indicator of environmental stress (2).

P. littoralis did not assimilate ¹³C readily, and consequently did not exhibit high bioconversion rates. *T. longicornis* displayed higher rates of *de novo* bioconversion for eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) than *P. littoralis* at all temperatures, with the exception of DHA at 23 °C. This temperature was the most stressful for the calanoid displaying a higher mortality with warming, comparatively the harpacticoid was eurythermal, with survival independent of temperature. The relative EPA, an important EFA, in *P. littoralis* did not change between the field and the experimental samples, therefore we hypothesize that this species is able to maintain the required amounts necessary, thus not needing to allocate energy towards these costly metabolic processes.

Although there may be a reduction in absolute omega-3 availability in primary producers, it is important to consider that complete absence, as in our experiment, is not a realistic scenario. Despite the fact that *T. longicornis* demonstrated higher *de novo* production, albeit not in sufficient amounts, individuals depleted their field EPA stores more rapidly. This indicates that *T. longicornis* is not able to biosynthesize EPA at a rate necessary for basic metabolic functioning, yet *P. littoralis* has maintained its relative stores, suggesting these extremes are within their coping capacity. Under the stressors imposed, *P. littoralis* has a greater potential for adaptive resilience when faced with extreme conditions in comparison to *T. longicornis*. This is the first experiment to quantify the *de novo* production of FA in the

dominant North Sea calanoid *T. longicornis* and monitor EFA *de novo* production along a thermal gradient in a comparative study with both harpacticoid and calanoid copepods.

References

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