LIPIDS BIOSYNTHESIS BY BENTHIC FORAMINIFERA IN OXIC VS. ANOXIC CONDITIONS: A NANOSIMS, GC/MS AND GC/C/IRMS STUDY

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Benthic foraminifera *Ammonia tepida* are ubiquitous in coastal marine sediments, where they are often exposed to hypoxia or completely anoxic conditions. In order to survive such anoxic conditions for longer time periods they must either rely on alternative, anaerobic metabolism (e.g. denitrification), which would allow them to produce energy and thus maintain a certain level of activity, or enter a state of dormancy that minimizes energy consumption. For other species, a state of highly reduced metabolism, essentially a state of dormancy, has been proposed but never demonstrated.

Here, we combined a 4 weeks feeding experiment, using ^{13}C-enriched diatom-containing biofilm, with correlated TEM and NanoSIMS imaging, plus measurements of concentrations and stable carbon isotope compositions of total organic matter and individual fatty acids (FAs), to study metabolic differences in the intertidal species *Ammonia tepida* exposed to oxic and anoxic conditions. Strongly contrasting cellular-level dynamics of integration and transfer of the ingested biofilm components were observed between the two conditions. Under oxic condition, within a few days, intact diatoms (i.e. including the frustule) were ingested, assimilated, and consumed, in part for biosynthesis of different cellular components: ^{13}C-labeled lipid droplets formed over a timescale of a few days and were then partly lost through respiration. In contrast, in anoxia, fewer diatoms were initially ingested and these were not assimilated or metabolized further, but remained visible within the foraminiferal cytoplasm even after 4 weeks.

Under oxic condition, the relative abundances of 16:0 and 18:1 isomers increased between 0 and 7 days (p<0.05), and remained stable between Day 7 and 28 (Fig. 1A). The relative abundances of 14:0 and 16:1(*n*-7) decreased between Days 0 (control) and 7 (p<0.05). Between Days 7 and 28, the relative abundance of 14:0 remained constant, while that of 16:1(*n*-7) continued to decrease. The abundance of 20:5(*n*-3) first decreased between Days 0 (control) and 7, and then increased to its highest level at Day 28 (p<0.05). Despite being present in small amounts in the diatom biofilm, the polyunsaturated FAs (PUFAs) 20:4(*n*-6) and 22:5(*n*-3) significantly increased in relative abundance along the experiment (p<0.05); most pronounced for 20:4(*n*-6) from 8.1 % in the control to 17.1 % (Fig. 1). Significant variation in the abundance of 22:6(*n*-3) was not observed during the experiment. Under anoxia, the relative variations in the abundance of individual FAs with time were significantly smaller than those observed under oxic condition. Only the abundance of 14:0 decreased slightly during the experiment, with a trend similar to that observed under oxic condition. No significant changes (p>0.05) were observed in the contents of 16:0, 22:5(*n*-3), and 22:6(*n*-3) during the experiment. 16:1(*n*-7) first increased slightly, then decreased from Day 7 to Day 28 (p<0.05). 18:1 abundance first decreased at Day 7 (p<0.05), and stabilized (p>0.05). 20:4(*n*-6) was the only FA that showed a significant, albeit minor increase (from

8.1±0.1 to 10.2±0.4 %; $p<0.05$) along the experiment. The ^{13}C atomic fraction of FAs ($x(^{13}C)_{FA}$ in %) were significantly higher after Day 7 of incubation ($p<0.05$) under both conditions; in general the ^{13}C-enrichments were higher under oxic than anoxic conditions. Under anoxia *A. tepida* ingested ^{13}C-enriched diatom biofilm only during Day 1. The observed increase during the first 7 days of 16:0 in oxic condition and of 16:1(*n*-7) in anoxic condition is ascribed to the ingestion of diatoms. The decreases of 14:0 in both conditions and of 16:1(*n*-7) in oxic condition at Day 7 suggest lipolysis and fatty acid catabolism (their β-oxidation to C_2 units). Part of the degradation products were probably used for *de novo* synthesis of long chain fatty acid intermediates for the production of PUFAs, *i.e.* 20:4(*n*-6), 20:5(*n*-3), and 22:5(*n*-3) in oxic condition. The metabolic behavior of eicosapentaenoic acid, 20:5(*n*-3), is particular: in oxic condition its relative abundance first decreased and then increased. This suggests that 20:5(*n*-3) was first consumed during metabolic breakdown or used for the synthesis of 22:5 and then formed by desaturation and C_2 elongation of short-chain precursors. The increase in relative content of 20:5(*n*-3) between Days 7 and 28 cannot be explained by an ingestion of diatoms, because the biofilm was completely ingested after Day 7 in oxic condition, which would support *de novo* synthesis of 20:5(*n*-3) by the foraminifera.

The PUFAs 20:4(*n*-6) and 22:5(*n*-3) were present only in small abundances in the diatom biofilm (< 2 %). Their high concentrations in *A. tepida* cytoplasm from both conditions could be due to either a selective uptake of these PUFAs (Ward et al., 2003), or by *de novo* biosynthesis following a pathway similar to that for 20:5(*n*-3). 20:4(*n-6*) was present in very low concentrations in the biofilm (0.02 %) but in relatively high concentrations in foraminifera. Its content increases significantly during the experiment in both oxic and anoxic conditions (Figure 1). The observed concentration increase, combined with significant ^{13}C-enrichment, strongly suggest *de novo* synthesis of arachidonic acid (20:4) by the foraminifera.

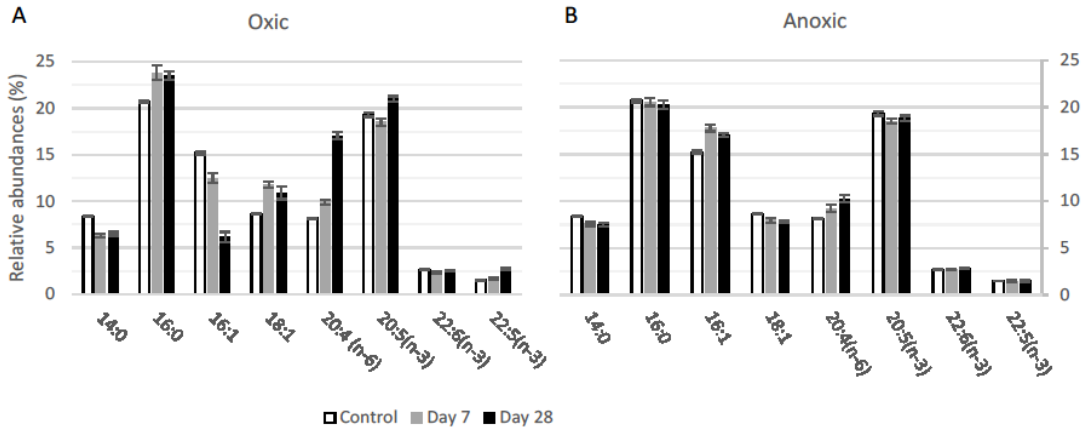

Figure 1 *Relative abundances (%) of FAs in A. tepida control specimens (white), after Day 7 (grey) and Day 28 (black) of incubation under oxic (A) and anoxic (B) conditions.*

Reference

Ward, J.N., Pond, D.W., Murray, J.W., 2003. Feeding of benthic foraminifera on diatoms and sewage-derived organic matter: an experimental application of lipid biomarker techniques. Mar. Environ. Res. 56, 515–30.