Pronounced uptake and metabolism of organic substrates by diatoms revealed by pulse-labeling metabolomics

Nils Meyer[1], Aljoscha Rydzyk[1], Georg Pohnert[1,2]*

[1]Institute for Inorganic and Analytical Chemistry, Bioorganic Analytics, Friedrich Schiller University Jena, Lessingstrasse 8, D-07743 Jena, Germany

[2]Max Planck Institute for Chemical Ecology, Hans Knöll Str. 8, D-07745 Jena, Germany

Supplementary Materials

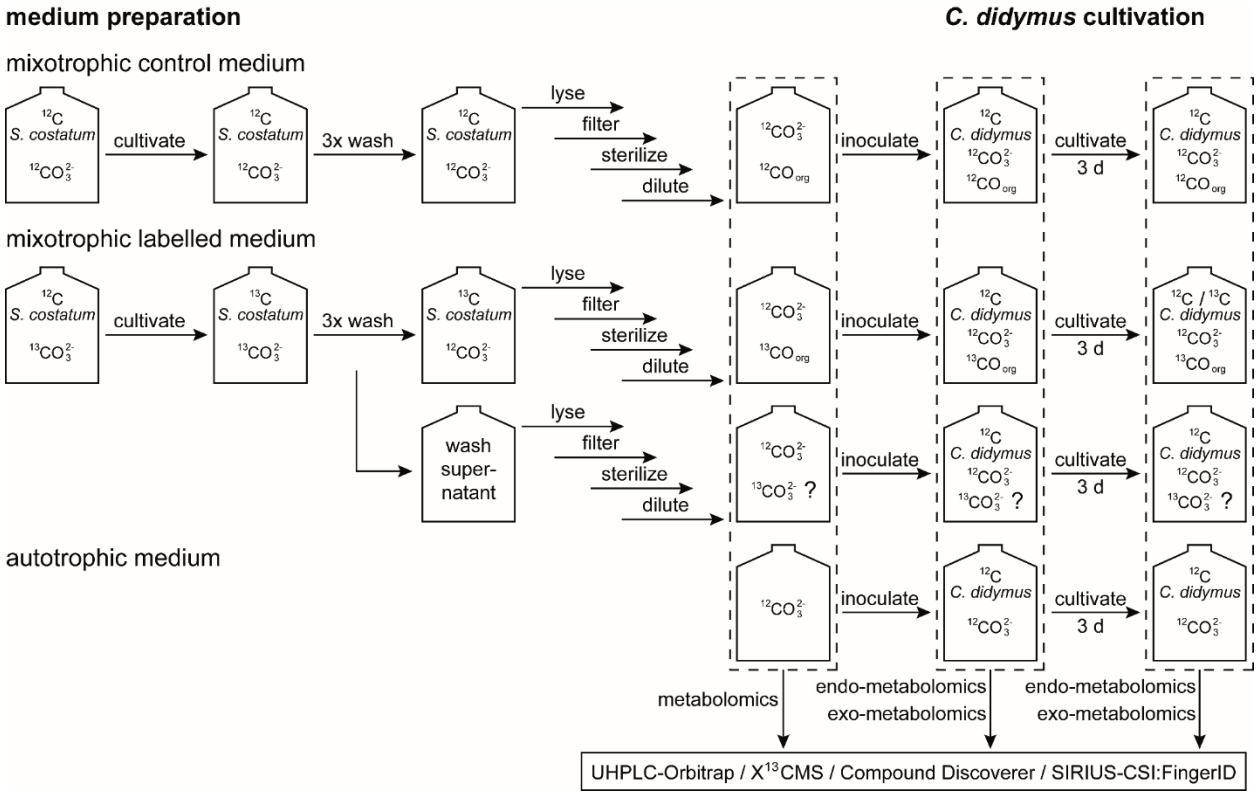

Fig. S1. Experimental setup for mixotrophy experiment. Last two lines: To demonstrate the effective removal of inorganic ^{13}C, *C. didymus* was grown on the wash supernatant and did not contain labelled metabolites

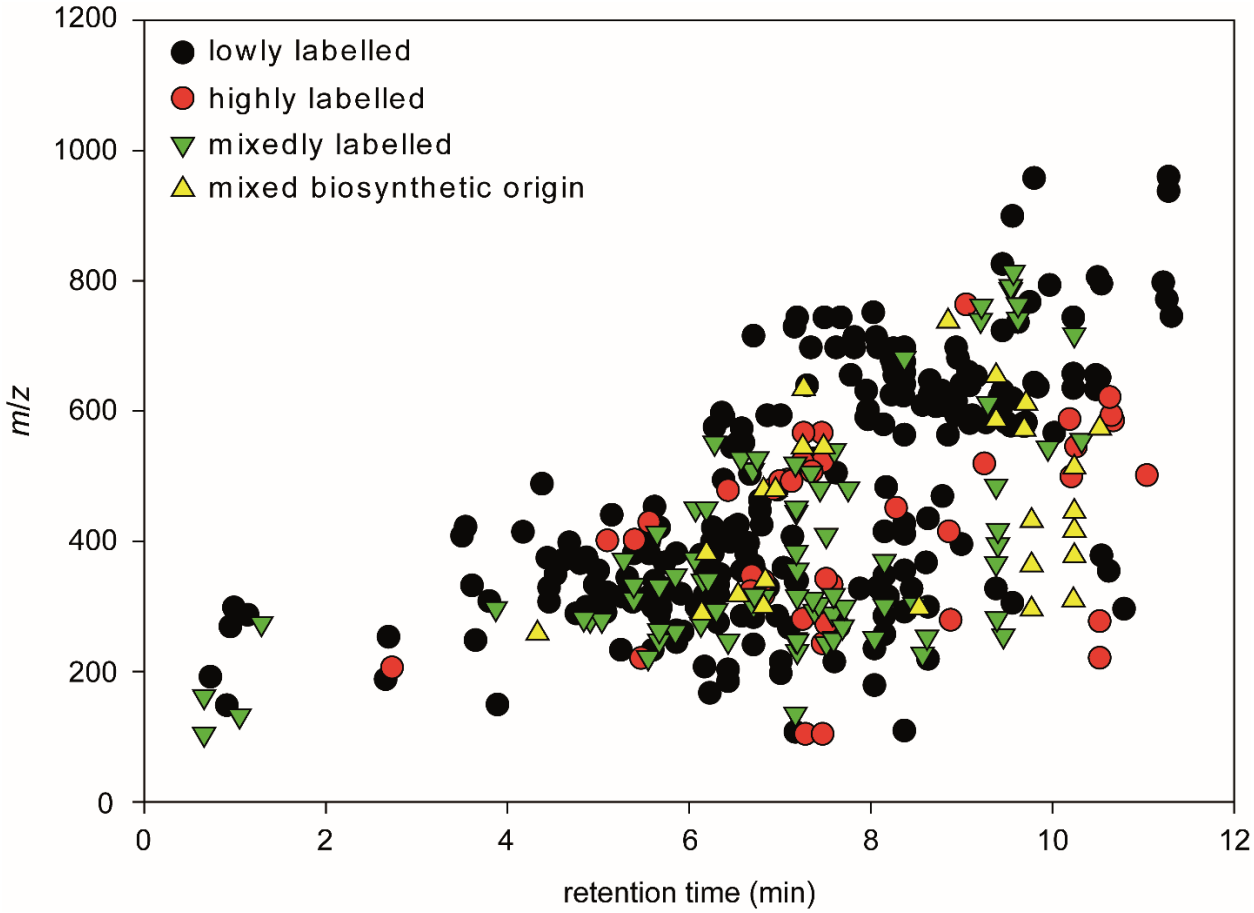

Fig. S2. Labelling of metabolites in *Chaetoceros didymus* endometabolome. Correlation between retention time (gradient as described in materials and methods) and *m*/*z* of manually curated isotopologue groups sorted by labelling pattern as described in Fig. 1.

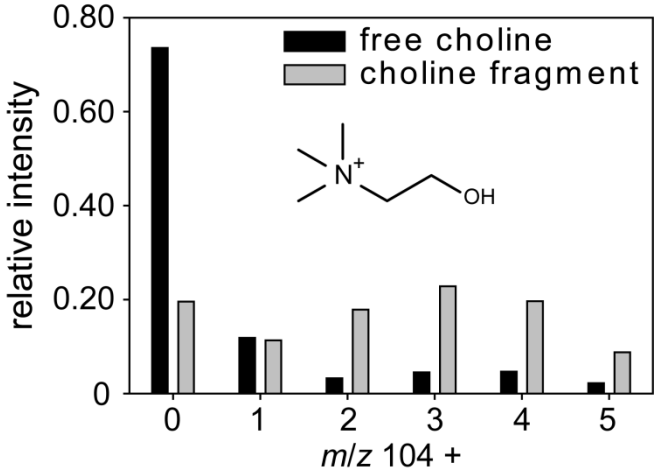

Fig. S3: Labelling pattern of choline. Depicted are the isotopologues of free choline and of the choline fragment from lysophosphatidylcholine in *C. didymus* exposed to labeld metabolites in positive ionization mode.

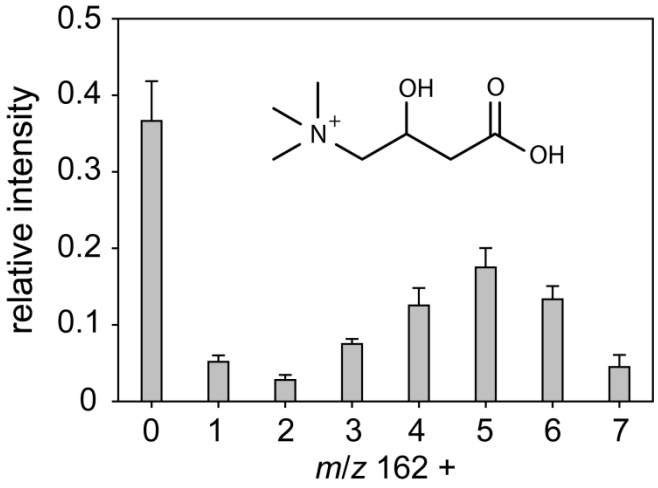

Fig. S4: Labelling pattern of carnitine. Depicted are the isotopologues of carnitine. The presence of M+5 to M+7 proves labelled *N*-methyl groups.

R-script for X^{13}CMS analysis

```
require(xcms)
require(X13CMS)
# set working directory to one where the "C12" and "C13" folders reside
setwd("E:/X13CMS")
# Peak-picking and retention-time alignment with XCMS
xs= xcmsSet(    c('./C12', './C13'), method= 'centWave', ppm= 3, peakwidth= c(5, 20))
xs= group(xs, bw=5, mzwid=0.015)
xs2= retcor(xs, method= 'obiwarp')
xs2= group(xs2, bw=5, mzwid=0.025)
xs3= fillPeaks(xs2)
# Setting variables for X13CMS
sN = rownames(xs3@phenoData) # sample names
sN = sN[c(1:3, 4:6)] # samples (3 unlabeled, 3 labeled)
# -----only significantly different isotopologues ------
# labeling report for samples:
labelsSign = getIsoLabelReport(xcmsSet = xs3, sampleNames = sN, unlabeledSamples = "C12", labeledSamples = "C13",
isotopeMassDiff = 1.00335, RTwindow = 10, ppm = 3, massOfLabeledAtom = 12, noiseCutoff = 10000, intChoice =
"intb", varEq = FALSE, alpha = 0.05, singleSample = FALSE, compareOnlyDistros = FALSE, monotonicityTol = FALSE,
enrichTol = 0.1)
# in each of the sN variables, the first 3 samples listed are of the "C12" or unlabeled type while the next 3 are of the "C13"
type
classes = c(rep("C12",3), rep("C13",3))
# print labeling report to a text file (recommended to open in Excel)
printIsoListOutputs(listReport = labelsSign, outputfile = "significant/labels_sign.txt")
# print pdf of isotopologue groups in a single labeling report plotted as relative intensity distributions
plotLabelReport(isoLabelReport = labelsSign, intOption = "rel", classes, labeledSamples = "C13", outputfile =
"significant/labelsrel_sign.pdf")
# print pdf of isotopologue groups in a single labeling report plotted as absolute intensity distributions
plotLabelReport(isoLabelReport = labelsSign, intOption = "abs", classes, labeledSamples = "C13", outputfile =
"significant/labelsabs_sign.pdf")
# -----all isotopologues ------
# labeling report for samples:
labelsAll = getIsoLabelReport(xcmsSet = xs3, sampleNames = sN, unlabeledSamples = "C12", labeledSamples = "C13",
isotopeMassDiff = 1.00335, RTwindow = 10, ppm = 3, massOfLabeledAtom = 12, noiseCutoff = 10000, intChoice =
"intb", varEq = FALSE, alpha = 1, singleSample = FALSE, compareOnlyDistros = FALSE, monotonicityTol = FALSE,
enrichTol = 0.1)
# in each of the sN variables, the first 3 samples listed are of the "C12" or unlabeled type while the next 3 are of the "C13"
type
classes = c(rep("C12",3), rep("C13",3))
# print labeling report to a text file (recommended to open in Excel)
printIsoListOutputs(listReport = labelsAll, outputfile = "all/labels_all.txt")
# print pdf of isotopologue groups in a single labeling report plotted as relative intensity distributions
plotLabelReport(isoLabelReport = labelsAll, intOption = "rel", classes, labeledSamples = "C13", outputfile =
"all/labelsrel_all.pdf")
# print pdf of isotopologue groups in a single labeling report plotted as absolute intensity distributions
plotLabelReport(isoLabelReport = labelsAll, intOption = "abs", classes, labeledSamples = "C13", outputfile =
"all/labelsabs_all.pdf")
```

Bernoulli statistics to calculate the degree of labelling

$$I(m) = \frac{n!}{m!\,(n-m)!} \cdot p^m \cdot (1-p)^{n-m}$$

For a metabolite with *n* carbon atoms the intensity of an isotopologue *I(m)* with *m* ^{13}C atoms is calculated using the degree of labelling *p*.