Potential biological sources of long chain alkyl diols in a lacustrine system

Laura Villanueva[a*], Marc Besseling[a], Marta Rodrigo-Gámiz[a], Sebastiaan W. Rampen[a], Dirk Verschuren[b], Jaap S. Sinninghe Damsté[a]

[a] *Royal Netherlands Institute for Sea Research, Department of Marine Organic Biogeochemistry, PO Box 59, 1790AB Den Burg, The Netherlands*

[b] *Limnology Unit, Ghent University, K. L. Ledeganckstraat 35, B-9000 Gent, Belgium*.

***** Corresponding author. *E mail address*: laura.villanueva@nioz.nl (L. Villanueva).

ABSTRACT

Long chain alkyl diols (LCDs) are lipids that have been detected in a wide range of marine and lacustrine environments, as well as in several algal cultures. However, the identity of the producers, their preferred ecological niche and seasonality are uncertain. We applied a gene-based approach to determine the identity and abundance of Eustigmatophyceae 18S rRNA genes and compared these data with the distribution of LCDs in the water column of Lake Challa (East Africa). Gene-based analysis revealed three known and two novel Eustigmatophyceae groups. Maxima in the number of gene copies and LCD concentration coincided at 9 m water depth, signifying Eustigmatophyceae as important producers of LCDs. In addition, seasonal

changes in LCD abundance in sedimenting particles revealed several blooms of LCD producers over the annual cycle.

Keywords: Long chain diols, eustigmatophytes, Lake Challa, Long chain Diol Index (LDI), gene-based approach.

1. Introduction

Long chain alkyl diols (LCDs) consist of an alkyl chain with OH groups at C-1 and at a mid-chain position. LCDs with 28–32 carbons atoms and OH groups at C-1,13 and C-1,15 have been found in Eustigmatophyceae cultures of marine (*Nannochloropsis* sp., *Eustigmatophyceae* sp.; Volkman et al., 1992) and freshwater species (*Vischeria* sp., *Eustigmatos* sp.; Volkman et al., 1999). Other sources outside the Eustigmatophyceae are some members of the *Proboscia* diatom genus (Sinninghe Damsté et al., 2003) and the alga *Apedinella radians* of the Dictyochophyceae phylum, both of which produce 1,14-diols (Rampen et al., 2011). LCDs have been found in marine and lacustrine sediments (e.g. Versteegh et al., 1997). Recently, Rampen et al. (2012) proposed the long chain diol index (LDI) as a novel marine paleotemperature proxy based on the C_{30} 1,15-diol abundance relative to the C_{28} 1,13-diol, and C_{30} 1,13-, 1,15-diols. The Eustigmatophyceae are generally considered to be major producers of LCDs in lakes but the identity of lacustrine LCD producers, their preferred niche in the water column, and their seasonality is uncertain. This information could improve the predictive power of the LDI proxy as we could anticipate spatial and seasonal biases influencing the reconstructed temperatures.

Here, we have developed a genetic-based approach to identify and quantify the abundance of potential LCD producers based on the 18S rRNA gene of members of the Eustigmatophyceae and its comparison with the distribution, abundance and seasonality of LCDs in a lake system.

2. Study site and sampling

Lake Challa is a permanently stratified crater lake on the southeastern flank of Mt. Kilimanjaro (East Africa). Suspended particulate matter (SPM) was collected at 5- and 10-m intervals throughout the water column in early February 2010 (see Buckles et al., 2013 for details and physicochemical conditions at the time of sampling); here we focus on samples comprised between 0.5 and 24 m depth, i.e. within and just below the photic zone. A mid-lake sediment trap at 35 m depth collected monthly samples of settling particles between from August 2009 to August 2010.

3. Material and methods

3.1. DNA methods

DNA was extracted from SPM filtered on GF/F 0.7 μm filters as described by Buckles et al. (2013). Primer pair Eust287F (5'- CGA CRA MTC ATT CAA GYT TCT GCC-3'), Eust810R (5'-CCA TGC TAR TGT ATT CAS GGC CT-3') was designed manually, and tested computationally and in PCRs. Gradient PCR was performed with melting temperature (Tm) ranging from 52−63 °C with genomic DNA extracted from different algal cultures (optimal Tm 58 °C). Quantitative PCR (qPCR) using the Eust287F/810R primer pair was performed at Tm of 61 °C and 45 cycles following the conditions described by

Buckles et al. (2013). A phylogenetic tree was inferred from the Neighbour-joining method and distances computed with the Jukes-Cantor method. Sequences NCBI accession numbers are KF765160 - KF765375.

3.2. Lipid methods

Filters from the SPM and the sediment trap were base hydrolyzed according to de Leeuw et al. (1983) by refluxing for 1 h with 1 N KOH in MeOH (96%). After cooling, the solvent was acidified with 2 N HCl/MeOH (1:1; v/v) to pH 2 and transferred to a separatory funnel. Thereafter, the filters were extracted using MeOH/H_2O (1:1 v/v; 1x), MeOH and dichloromethane (DCM; 3x). Solvent was collected in a separatory funnel containing ca. 25 ml bidistilled H_2O. The DCM layer was separated from the H_2O/MeOH layer and the remaining H_2O/MeOH layer was extracted (3x) with DCM. The extracts were combined and rotary evaporated to near dryness. The resulting extract and the residual filters were hydrolyzed with acid (3 h reflux, 2 N HCl/MeOH, 1:1; v/v) and neutralized with 1 N KOH in MeOH (96%). Filters were extracted as above while for the extracts, 3 ml bidistilled H_2O was added and the lipids extracted using DCM (4x). All extracts were combined, dried under N_2, eluted in DCM over a pipette column containing Na_2SO_4, dried under N_2, methylated in DCM using CH_2N_2 in Et_2O and dried under N_2. An internal standard C_{22} 7,16-diol was added to the total lipid extracts and each extracts were fractionated into apolar and polar fractions using a glass pipette column with activated Al_2O_3 and eluted with hexane/DCM (9/1; v/v) and DCM/MeOH (1/1; v/v). Each polar fraction was

silylated prior to gas chromatography-mass spectrometry (GC-MS). LCD analysis was carried out as described by Rampen et al. (2012).

4. Results and discussion

4.1. Eustigmatophyceae and LCD diversity and abundance

In order to determine eustigmatophyte diversity contained in Lake Challa SPM, clone libraries were generated by cloning 18S rRNA gene fragments generated by the primers Eust287F/Eust810R. Sequences from 0.5, 9, and 19 m water depth all clustered into five distinctive phylogenetic groups (Fig. 1). No clustering of sequences according to depth was observed as those recovered from the three depths were distributed throughout the tree. Group 1 sequences were closely related to those sequences of the *Goniochloridaceae* family (Pribyl et al., 2012), while groups 4 and 5 sequences clustered with sequences of the *Monodopsidaceae* and *Eustigmataceae* families. Sequences falling in groups 2 and 3 diverged from sequences of cultured representatives, supporting their assignment to one or more unknown Eustigmatophyceae families. Quantification of Eustigmatophyceae gene copies showed a distinctive peak at 9 m depth (Fig. 2A). The most abundant LCDs in the February SPM samples were C_{32} 1,15 (138 ng l^{-1}), C_{30} 1,15 (54 ng l^{-1}), and C_{34} 1,17-diols (23 ng l^{-1}). Of these, the C_{34} 1,17-diols may be produced by the novel Eustigmatophyceae with group 2 and 3 sequences, since these diols have previously been found in lake samples (Versteegh et al., 1997; Zhang et al., 2011), but have not been detected in freshwater eustigmatophyte cultures (Volkman et al., 1999).

Maximum LCD abundance was at 9 m (62 ng l^{-1}; Table 1, Fig. 2B), coinciding with the maximum abundance of Eustigmatophyceae 18S rRNA gene copies (Fig. 2). This correlation supports the Eustigmatophyceae as important LCD producers in this lake system. High LCD abundance (38–46 ng l^{-1}) coincides with little or no Eustigmatophyceae 18S rRNA gene copies in the uppermost part of the water column (0–5 m). This pattern may be explained by wind-driven and convective mixing of preserved LCDs throughout the epilimnion, whereas living algal cells adjust their buoyancy to their preferred habitat at slightly greater depth.

4.2. Seasonality of LCDs

Peak LCD fluxes in descending particles were detected in February, April and June 2010 (Table 2), with C_{32} 1,15, C_{30} 1,15, and C_{34} 1,17-diols accounting for >85% of total LCD abundance. LCD in settling particles during February (Table 2) was similar to that found in the SPM on early February (Table 1). In April, the most abundant LCD was the C_{30} 1,15-diol, while in February and June it was the C_{32} 1,15-diol. These differences in the relative abundance of individual LCDs in February and June vs. April may reflect temporary blooms of different LCD producers or a change in the distribution of LCDs within the same producer. Successive seasonal blooming of different Eustigmatophyceae could indicate niche separation controlled by temperature variation in the upper water column (peaking at ca. 27 ºC in February), or seasonal nutrient dynamics influenced by the timing of rainfall and water column stratification.

5. Conclusions

The application of a 18S rRNA gene-based method has revealed the presence of both known and novel groups of Eustigmatophyceae in Lake Challa. Maximum abundance of Eustigmatophyceae gene sequences coincided with maximum LCD abundance at 9 m water depth, suggesting an important role of eustigmatophytes as LCD producers. Seasonal variation in LCD distributions suggests that successive LCD-producing blooms are due to different eustigmatophyte algae or changes in the LCDs produced by a unique algal population in evolving abiotic conditions.

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189

190 **Table 1**

191 Quantification of LCDs (ng/l filtered) in Lake Challa SPM samples collected
192 in February 2010.
193

Depth (m)	C_{30} 1,14	C_{30} 1,15	C_{30} 1,16	C_{32} 1,15	C_{32} 1,16	C_{34} 1,15	C_{34} 1,17	Total
0.5	0.5	9.8	0.9	29.6	0.5	0.0	4.4	46
4	0.6	9.5	1.1	22.9	0.4	0.0	3.6	38
9	1.0	14.9	0.0	38.1	0.0	0.4	7.3	62
14	0.8	12.9	0.0	32.2	0.0	0.4	5.1	52
19	0.0	5.2	0.0	10.9	0.0	0.0	1.9	18
24	0.0	2.1	0.0	4.1	0.0	0.0	0.6	6.9

194

195 **Table 2**

196 LCD flux (μg/m²/day) for particles settling in a mid-lake sediment trap in
197 Lake Challa.
198

Date	C_{30} 1,13	C_{30} 1,14	C_{30} 1,15	C_{31} 1,15	$C_{32:1}$ 1,15	C_{32} 1,13	C_{32} 1,15	C_{32} 1,16	C_{34} 1,15	C_{34} 1,17	Total
Aug'09	0.2	1.1	3.8	0.3	0.0	0.0	3.8	0.1	0.0	0.6	9.9
Sep'09	1.8	2.7	5.3	1.4	0.6	0.0	9.5	0.2	0.0	0.8	22
Oct'09	0.3	0.5	2.9	0.1	0.0	0.0	1.0	0.0	0.0	0.2	5.0
Nov'09	1.9	1.8	23.0	1.5	0.2	0.0	9.4	0.3	0.0	0.6	39
Dec'09	0.6	1.5	18.4	0.5	0.3	0.0	7.6	0.3	0.0	1.8	31
Jan'10	0.1	0.3	3.5	0.2	0.0	0.2	10.0	0.3	0.0	3.3	18

Feb'10	0.3	2.5	35.0	2.2	0.6	1.6	83.7	2.1	0.5	35.3	165
Mar'10	2.4	3.5	38.1	1.6	0.2	0.2	26.1	0.9	0.1	6.1	79
Apr'10	10.1	11.2	111.8	5.1	0.7	0.4	48.2	2.3	0.1	16.1	206
May'10	1.5	2.3	25.0	0.9	0.2	0.2	23.3	0.6	0.1	7.4	62
Jun'10	1.3	2.3	20.0	1.8	0.4	0.4	57.4	0.8	0.2	22.5	107
July'10	0.6	2.0	10.9	0.6	0.2	0.0	15.2	0.3	0.0	5.7	36
Aug'10	0.0	1.5	5.8	0.4	0.0	0.0	8.7	0.0	0.0	3.2	20

Fig. 1. Phylogenetic tree for 18S rRNA gene sequences recovered, and closest relatives in the Eustigmatophyceae phylum. Branch support (in %) is indicated on the branches. Scale bar indicates 0.02 substitutions per site. Letter and number code, e.g. 19 m E3 is an arbitrary code assignation to the sequences recovered after cloning.

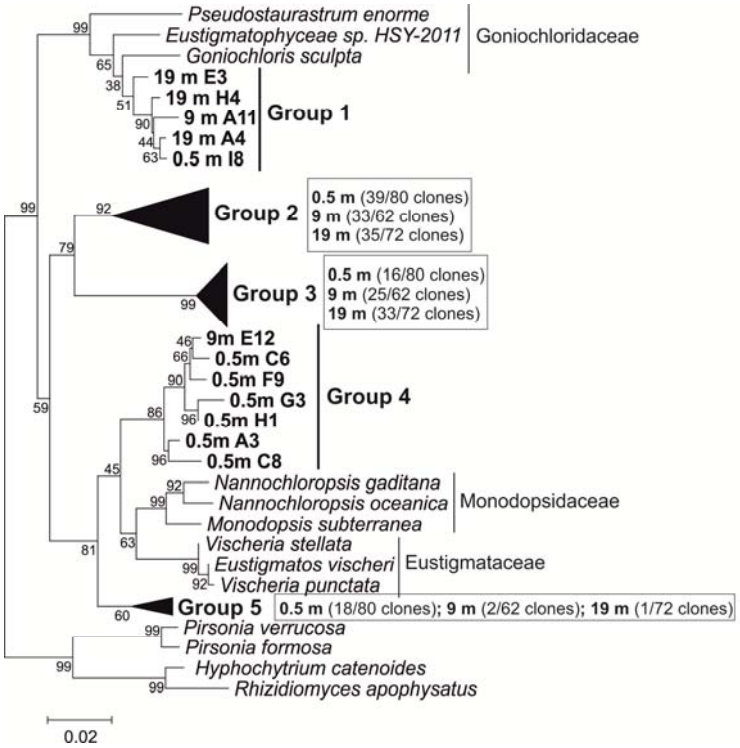

Fig. 2. Quantification of Eustigmatophyceae 18S rRNA gene copies and total LCDs in SPM from the upper water column of Lake Challa collected in early February 2010.