

1 Seasonal lake surface water temperature trends reflected 2 by heterocyst glycolipid based molecular thermometers

3
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10 11 **Abstract**

12 It has been demonstrated that the relative distribution of heterocyst glycolipids (HGs) in
13 cultures of N₂-fixing heterocystous cyanobacteria is largely controlled by growth temperature,
14 suggesting a potential use of these components in paleoenvironmental studies. Here, we
15 investigated the effect of environmental parameters (e.g. surface water temperatures, oxygen
16 concentrations and pH) on the distribution of HGs in a natural system using water column
17 filtrates collected from Lake Schreventeich (Kiel, Germany) from late July to the end of
18 October 2013. HPLC-ESI/MS analysis revealed a dominance of 1-(O-hexose)-3,25-
19 hexacosanediols (HG₂₆ diols) and 1-(O-hexose)-3-keto-25-hexacosanol (HG₂₆ keto-ol) in the
20 solvent extracted water column filtrates, which were accompanied by minor abundances of 1-
21 (O-hexose)-3,27-octacosanediol (HG₂₈ diol) and 1-(O-hexose)-3-keto-27-octacosanol (HG₂₈
22 keto-ol) as well as 1-(O-hexose)-3,25,27-octacosanetriol (HG₂₈ triol) and 1-(O-hexose)-3-
23 keto-25,27-octacosanediol (HG₂₈ keto-diol). Fractional abundances of alcoholic and ketonic
24 HGs generally showed strong linear correlations with surface water temperatures and no or
25 only weak linear correlations with both oxygen concentrations and pH. Changes in the
26 distribution of the most abundant diol and keto-ol (e.g., HG₂₆ diol and HG₂₆ keto-ol) were
27 quantitatively expressed as the HDI₂₆ (heterocyst diol index of 26 carbon atoms) with values
28 of this index ranging from 0.89 in mid-August to 0.66 in mid-October. An average HDI₂₆
29 value of 0.79, which translates into a calculated surface water temperature of 15.8±0.3°C, was
30 obtained from surface sediments collected from Lake Schreventeich. This temperature - and

1 temperatures obtained from other HG indices (e.g., HDI₂₈ and HTI₂₈) - is similar to the one
2 measured during maximum cyanobacterial productivity in early to mid-September and
3 suggests that HGs preserved in the sediment record of Lake Schreventeich reflect summer
4 surface water temperatures. As N₂-fixing heterocystous cyanobacteria are widespread in
5 present-day freshwater and brackish environments, we conclude that the distribution of HGs
6 in sediments may allow the reconstruction of surface water temperatures of modern and
7 potentially ancient lacustrine settings.

8

9 **1 Introduction**

10 Lipid paleothermometers have become an indispensable tool in paleoenvironmental studies as
11 they allow the reconstruction of oceanic surface water temperatures over geological time
12 scales and thus provide essential information on past climate changes. The two most
13 commonly employed lipid paleothermometers are the U^K₃₇ (Brassell et al., 1986) and the
14 TEX₈₆ (Schouten et al., 2002), which use the distribution of long chain alkenones or glycerol
15 dialkyl glycerol tetraether (GDGT) preserved in marine sediments to reconstruct oceanic
16 surface water temperatures. The more recently introduced long chain diol index (LDI), which
17 is based on the distribution of C₂₈ 1,13-, C₃₀ 1,13-, and C₃₀ 1,15-diols produced by
18 eustigmatophyte algae (Rampen et al., 2012), provides an additional mean to determine past
19 changes in sea surface temperatures (SST) and has successfully been applied in a number of
20 paleoceanographic studies (Smith et al., 2013; Rodrigo-Gámiz et al., 2014).

21 The TEX₈₆ proxy has previously been applied to a number of freshwater environments but
22 seems to reliably predict surface water temperatures only in some large lakes, such as the
23 North American Great Lakes and the African Rift Valley lakes, where the contribution of
24 isoprenoid GDGTs of a terrestrial origin is only negligible (Powers et al., 2010). Likewise,
25 long chain alkenones have been reported from some modern lake systems (Volkman et al.,
26 1988; Thiel et al., 1997; Theroux et al., 2012) and were employed to reconstruct past changes
27 in surface water temperatures in Lake Steisslingen, SW Germany (Zink et al., 2001).
28 However, due to our incomplete knowledge on the biological sources of long chain alkenones
29 and their comparatively limited distribution in freshwater environments, temperature
30 estimates based on long chain alkenones in lacustrine sediments are comparatively few.

31 Another lipid paleothermometer that has attracted considerable attention over the recent past
32 is the MBT (methylation index of branched tetraethers)/CBT (cyclisation ratio of branched

1 tetraethers) index. This proxy, based on the distribution of branched GDGTs that are
2 ubiquitously distributed in soils, peats as well as lacustrine and coastal marine sediments (see
3 Schouten et al., 2013 and references therein), has been shown to correlate well with mean
4 annual air temperature (MAAT) and soil pH (Weijers et al., 2007). It has since also been
5 applied to various lakes and coastal marine environments, containing a large proportion of
6 terrestrial organic matter, to infer past changes in continental climate (Zink et al., 2010;
7 Niemann et al., 2012; Berke et al., 2014). However, a number of more recent studies have
8 demonstrated that branched GDGTs may also be produced in aquatic systems, possibly
9 complicating the application of the MBT/CBT index as a tool for reconstructing MAAT (De
10 Jonge et al., 2014; Weber et al., 2015). Nonetheless, the MBT/CBT index and other lipid
11 paleothermometers have proven most valuable in determining trends in climate evolution both
12 on regional and global scales. A lipid-based proxy that allows deciphering past changes in
13 surface water temperatures in lacustrine environments on the contrary is currently missing
14 (Castañeda and Schouten, 2011).

15 Heterocystous cyanobacteria are oxygenic photoautotrophs that are known to be an abundant
16 component of the phytoplankton community of many present-day freshwater lakes of polar to
17 tropical latitudes (Whitton, 2012). They are also known to form massive blooms in river
18 deltas and semi-enclosed basins such as the Baltic Sea (Stal et al., 1999; Larsson et al., 2001).
19 Their dominant role in the primary production of freshwater and brackish environments is
20 related to their unique ability to simultaneously perform oxygenic photosynthesis and
21 nitrogen fixation, enabling them to outcompete eukaryotic algae under nitrogen limiting
22 conditions (Levine and Schindler, 1999). For this, heterocystous cyanobacteria confine the
23 fixation of N₂ to heterocysts, which host the oxygen-sensitive enzyme nitrogenase that
24 catalyzes the reduction of dinitrogen gas to ammonia. These specialized cells are enveloped in
25 a set of unique glycolipids, so-called heterocyst glycolipids (HGs), which are exclusively
26 present in N₂-fixing heterocystous cyanobacteria (Nichols and Wood, 1968; Gambacorta et
27 al., 1999; Bauersachs et al., 2009a) and are considered to act as a gas diffusion barrier that
28 limits the entry of oxygen into the heterocyst (Wolk, 1982). These components are composed
29 of sugar head groups that are glycosidically bound to long chain diols, triols, keto-ols or keto-
30 diols with an even carbon chain ranging from C₂₆ to C₃₂ carbon atoms (Fig. 1). The
31 distribution of HG diols and keto-ols has previously been shown to strongly correlate with
32 growth temperature in cultures of the heterocystous cyanobacteria *Anabaena* CCY9613 and
33 *Nostoc* CCY9926 (Bauersachs et al., 2009a; 2014). These authors demonstrated that in both

1 types of cyanobacteria the relative proportion of HG diols significantly increased compared to
2 their corresponding HG keto-ols with increasing growth temperature and introduced the HG₂₆
3 (*heterocyst glycolipid index of 26 carbon atoms*) and HG₂₈ (*heterocyst glycolipid index of 28*
4 *carbon atoms*) as means to quantify structural changes in the HG composition of the
5 heterocyst cell envelope. It should be pointed out though that the overall change in the
6 structural composition of the heterocyst cell envelope varied significantly between both
7 cyanobacteria with HG₂₆ values varying from 0.10 to 0.18 in *Anabaena* CCY9613 and from
8 0.12 to 0.30 in *Nostoc* CCY9926 (Bauersachs et al. 2014), indicating that individual species
9 of heterocystous cyanobacteria may tune the properties of the gas diffusion barrier in a
10 slightly different fashion. Nonetheless, the finding of temperature induced changes in the
11 heterocyst glycolipid composition of N₂-fixing heterocystous cyanobacteria may offer the
12 exciting possibility to reconstruct surface water temperatures of modern and possibly also
13 fossil lacustrine environments given that (1) heterocystous cyanobacteria are a common
14 component of the phytoplankton community in many contemporary and fossil freshwater
15 environments (Whitton, 2012) and (2) HGs have been shown to preserve well in the
16 geological record (Bauersachs et al., 2010). Here, we investigated temporal variations in the
17 distribution of heterocyst glycolipids in water column filtrates of Lake Schreventeich (Kiel,
18 Germany). We also analyzed the distribution of HGs in surface sediments of this small
19 holomictic lake and discuss the potential use of HGs in the reconstruction of surface water
20 temperatures in modern and fossil freshwater environments.

21

22 **2 Material and methods**

23 **2.1 Study site and sampling**

24 Lake Schreventeich is a small holomictic lake situated in northern Germany (54°19'36.79"N,
25 10°07'17.57"E). Its surface area covers approximately 0.38 km² and it has an average depth
26 of 1.4-1.6 m (maximum depth of 3.4 m). The lake has no tributaries and is solely fed by
27 precipitation and ground water inflow.

28 Surface water samples for the analysis of HGs were taken from late July to the end of October
29 2013. Oxygen concentrations and surface water temperatures were measured at time of
30 sampling using the portable oxygen measuring instrument "Oxi 1970i" coupled to a
31 "CellOx325" oxygen probe (WTW, Germany). The pH of all water samples was determined

1 using a “FG2-FiveGo” (Mettler-Toledo, Germany) using a two-point calibration on certified
2 reference solutions obtained from Hanna Instruments. Surface sediments (0-1 cm) from two
3 locations within Lake Schreventeich were obtained in March 2014 using an Uwitech gravity
4 corer (Uwitech, Switzerland). All sediments were freeze-dried and ground to a homogenous
5 powder using pestle and mortar.

6 **2.2 Determination of algal biomass**

7 100 mL of surface water were collected during each sampling and filtered over a preweighed
8 Whatman filter GF/C (1.2 μm , diameter 47 mm). After filtration, filters were manually
9 inspected and non-phytoplankton biomass was removed using a pair of tweezers. All filters
10 were subsequently dried in an oven at 105 °C for 24 hours. Phytoplankton biomass was
11 calculated as the weight difference between the preweighed and the oven-dried filters.

12 **2.3 Bligh and Dyer extraction of water column filtrates and core top** 13 **sediments**

14 Measured volumes (e.g., 3-4 L) of surface water were filtered through a MN 85/70 BF glass
15 fiber filter with a pore size of 0.45 μm (Macherey-Nagel, Germany). All filters were freeze-
16 dried and extracted following a modified Bligh and Dyer procedure as described by Rütters et
17 al. (2002). Briefly, filters were cut into fine pieces with a solvent-cleaned scissor and
18 ultrasonically extracted using a solvent mixture of methanol (MeOH), dichloromethane
19 (DCM) and phosphate buffer (2/1/0.8; v/v/v). After centrifugation, the supernatant was
20 collected and the residue extracted twice with the solvent mixture specified above. DCM and
21 phosphate buffer were added to the pooled supernatants to achieve a ratio of
22 MeOH/DCM/phosphate buffer of 1:1:0.9 (v/v/v), allowing separation of two phases. The
23 bottom layer, containing the organic fraction, was transferred to a glass vial and the remaining
24 aqueous phase was extracted twice with DCM. The combined extracts were reduced under
25 rotary vacuum, transferred to preweighed vials and dried under a gentle stream of N_2 . All
26 Bligh and Dyer extracts were subsequently dissolved in DCM:MeOH (9:1; v/v) to a
27 concentration of 2 to 4 mg mL^{-1} and filtered through a 0.45- μm -pore-size regenerated
28 cellulose filter (13 mm; LLG Labware, Germany) prior to analysis. In addition to water
29 column filtrates, 0.5 gram of freeze-dried core top sediments obtained from Lake
30 Schreventeich were extracted using the procedure outlined above.

1 2.4 Analysis of heterocyst glycolipids

2 Heterocyst glycolipids were analyzed following the procedure described by Bauersachs et al.
3 (2014) with some brief modifications. Separation of the target compounds was achieved using
4 an Alliance 2690 HPLC system (Waters, UK) fitted with a Luna Hilic 200A column (150 mm
5 x 2 mm i.d.; 3 μ m; Phenomenex, Germany) maintained at 30 °C. The following linear
6 gradient was used with a flow rate of 0.2 mL min⁻¹: 95% eluent A/5% eluent B to 70% A/30%
7 B in 10 min (held 20 min), followed by 70% A/30% B to 35% A/65% B in 15 min (held 15
8 min), then back to 95% A/5% B in 1 min (held 20 min) to re-equilibrate the column. Eluent A
9 was hexane-isopropanol-formic acid-14.8 M aqueous NH₃ (79:20:0.12:0.04; v/v/v/v) and
10 eluent B was isopropanol-water-formic acid-14.8 M aqueous NH₃ (88:10:0.12:0.4; v/v/v/v).

11 Detection of heterocyst glycolipids was accomplished using a Quattro LC triple quadrupole
12 mass spectrometer (Micromass, UK). The positive electrospray ionization (ESI) conditions
13 were as follows: capillary voltage, 3.2 kV; cone voltage, 25 V; source temperature 120 °C;
14 desolvation temperature, 200 °C; cone gas flow, 1 L min⁻¹ and desolvation gas flow, 4 L min⁻¹.
15 To qualitatively determine the distribution of HGs in water column filtrates of Lake
16 Schreventeich, all Bligh and Dyer extracts were analyzed in data dependent mode with two
17 scan events, where a positive ion scan (m/z 300-1000) was followed by a product ion scan of
18 the base peak of the mass spectrum of the first scan event. Identification of HGs was based on
19 comparison with published mass spectra (Bauersachs et al., 2009b). To improve the
20 sensitivity of the measurement and therewith increase reproducibility, HGs were also detected
21 via single ion recording (SIR) of their protonated molecules [M+H]⁺ (dwell time 234 ms) with
22 m/z 575.5 (HG₂₆ keto-ol), m/z 577.5 (HG₂₆ diol), m/z 603.5 (HG₂₈ keto-ol), m/z 605.5 (HG₂₈
23 diol), m/z 619.5 (HG₂₈ keto-diol) and m/z 621.5 (HG₂₈ triol). Selected samples were analyzed
24 in duplicate and fractional abundances of HGs as well as calculated HG ratios (e.g., HDI₂₆,
25 HDI₂₈, HTI₂₈) given in the text represent average values of these measurements.
26 Quantification was done by integration of the peak area using the QuanLynx application
27 manager.

28

1 **3 Results**

2 **3.1 Variation of environmental parameters and algal biomass in Lake** 3 **Schreventeich**

4 Physical and biological data of Lake Schreventeich collected from late July to the end of
5 October 2013 are summarized in Figure 2. All investigated physical parameters (i.e.,
6 temperature, oxygen concentration and pH) show maxima in late July or at the beginning of
7 August and gradually decline to yield minima in late October. Surface water temperatures
8 ranged from 10.5 to 24.0°C and were highest in late July (Fig. 2a). Oxygen concentrations in
9 the surface waters ranged from 2.5 to 7.6 mg L⁻¹ with highest values occurring in late July and
10 they subsequently declined over the investigated time interval to yield minimum values in late
11 October (Fig. 2b). pH values ranged from 7.18 to 7.79 and were comparatively high during
12 the first half of the sampling campaign with values averaging 7.56 in August (Fig 2c). In
13 contrast, the pH showed a significant drop by almost 0.2 units at the beginning of September
14 and stayed around 7.32 throughout the first half of September before increasing again to
15 values of ca. 7.50 at the beginning of October. Lake productivity was determined by
16 measuring the amount of biomass present at time of sampling. Comparatively low amounts of
17 biomass were found in late July with values of 11.6 mg L⁻¹ that almost doubled in August
18 with an average value of 20.7 mg L⁻¹ (Fig. 2d). After a pronounced peak in the first half of
19 September (maximum 50.1 mg L⁻¹; average 35.3 mg L⁻¹), biomass concentrations declined to
20 an average value of 22.5 mg L⁻¹ in October.

21 **3.2 Distribution and fractional abundances of heterocyst glycolipids in water** 22 **column filtrates of Lake Schreventeich**

23 Heterocyst glycolipids were below detection limit in late July and early August. They were
24 first identified in mid-August in low relative abundances, gradually increased in late August
25 to reach peak abundances in early to mid-September (Fig. 3). In late September, the relative
26 abundance of HGs declined to reach comparatively low but constant values from mid- to late
27 October. As shown in Fig. S1 in the Supplement, two structural isomers of 1-(O-hexose)-
28 3,25-hexacosanediol (HG₂₆ diol) and 1-(O-hexose)-3-keto-25-hexacosanol (HG₂₆ keto-ol)
29 generally dominated the HG pool and together they constituted 71 to 100% (average 82.7 ±
30 7.2%) of all heterocyst glycolipids over the investigated time interval. The early eluting HG₂₆
31 diol, however, generally constituted only a minute fraction of all HGs (on average <0.5%).

1 The heterocyst glycolipids 1-(O-hexose)-3,25,27-octacosanetriol (HG₂₈ triol) and 1-(O-
2 hexose)-3-keto-25,27-octacosanediol (HG₂₈ keto-diol) were particularly abundant in late
3 August with fractional abundances of up to 25% but in general they contributed 6 to 17%
4 (average $12.3 \pm 6.2\%$) to the heterocyst glycolipid content of Lake Schreventeich. 1-(O-
5 hexose)-3,27-octacosanediol (HG₂₈ diol) and 1-(O-hexose)-3-keto-27-octacosanol (HG₂₈
6 keto-ol) were below detection limit in water column filtrates taken before early September
7 (Fig. 3) and they usually constituted a minor component of the total HG pool with fractional
8 abundances of both compounds ranging from 0 to 13% (average $4.9 \pm 3.8\%$).

9 It is interesting to note that the fractional abundance of all HG diols and triols declined over
10 the investigated time interval, while the fractional abundance of their corresponding keto-ol
11 and keto-diol varieties showed a concomitant increase (Fig. 3). For example, the fractional
12 abundance of the HG₂₆ diol was highest (>70%) in late August to early September and
13 thereafter declined gradually to yield values around 50% at the end of October. Over the same
14 time period, the fractional abundance of the HG₂₆ keto-ol significantly increased from 9% at
15 the end of August to 25% in late October. Overall similar trends were also observed for the
16 HG₂₈ triol and HG₂₈ keto-diol as well as for the HG₂₈ diol and HG₂₈ keto-ol. It should be
17 pointed out though that for the HG₂₈ diol and the HG₂₈ keto-ol this trend was less apparent,
18 which may be due to the low analytical response and the resulting uncertainties in
19 determining the contribution of both components to the total HG pool.

20 **3.3 Distribution and fractional abundances of heterocyst glycolipids in** 21 **surface sediments of Lake Schreventeich**

22 The distribution of HGs in surface sediments of Lake Schreventeich largely resembled those
23 observed in the water column filtrates with the HG₂₆ diol and HG₂₆ keto-ol being most
24 abundant (Fig. 3). Both components constituted ca. 81% of the total HG pool with HG₂₆ diol
25 and HG₂₆ keto-ol accounting for 64% and 17% of all HGs, respectively. HG₂₈ triol and HG₂₈
26 keto-diol were the second most abundant types of HGs in Lake Schreventeich sediments
27 contributing 7% and 4% of all HGs. Similar to the distribution of HGs in the water column
28 filtrates, HG₂₈ diol and HG₂₈ keto-ol constituted only a minor component of the HG pool with
29 5% and 3%, respectively. Fractional abundances of HGs found in the core top sediments of
30 Lake Schreventeich are thus well in line with those observed in water column filtrates; in
31 particular with those obtained in mid-September.

1

2 **4 Discussion**

3 **4.1 Sources and environmental controls on the HG distribution in water** 4 **column filtrates of Lake Schreventeich**

5 Heterocyst glycolipids were below detection limit in water column filtrates collected
6 throughout July to early August and were first observed in mid-August. In general, the
7 distribution of heterocyst glycolipids in Lake Schreventeich is well in line with those
8 previously reported from nostocalean cyanobacteria (Bauersachs et al., 2009a; Wörmer et al.
9 2012) and likely suggests that members of the genera *Anabaena* and/or *Aphanizomenon* were
10 part of the phytoplankton community of Lake Schreventeich in late summer 2013. This agrees
11 well with microbiological studies of the phytoplankton community of other North German
12 lakes, for which representatives of both genera have indeed been reported in abundance (Arp
13 et al., 2013). The simultaneous increase in total HG abundances and aquatic biomass in early
14 to mid-September (Figs. 2 and 3) may also suggest that heterocystous cyanobacteria
15 constituted a significant component of the lake's phytoplankton.

16 We observed systematic changes in the distribution of heterocyst glycolipids in water column
17 filtrates of Lake Schreventeich over the time interval investigated. The most apparent was a
18 systematic decline in the fractional abundances of HG diols and the HG triol from mid-
19 August to late October, which was significantly positively correlated with surface water
20 temperature (Table 1). On the contrary, fractional abundances of HG keto-ols and keto-diols
21 gradually increased from late August to the end of the sampling campaign. This increase in
22 fractional abundances was significantly negatively correlated with changes in surface water
23 temperatures (Table 1). Similar changes in the fractional abundances of HG₂₆ and HG₂₈ diols
24 and keto-ols with growth temperature have previously been described from cultures of the N₂-
25 fixing heterocystous cyanobacteria *Anabaena* CCY9613 and *Nostoc* CCY9926 (Bauersachs et
26 al., 2009a; 2014) and been explained as a physiological adaptation to compensate for greater
27 gas diffusion rates of O₂ at higher temperatures in order to keep the entry of atmospheric
28 gases into the heterocyst at a minimum, which is considered a prerequisite for optimum N₂
29 fixation. To quantitatively express these structural changes of the heterocyst cell envelope,
30 Bauersachs et al. (2009a) introduced the HG₂₆-index (*heterocyst glycolipid index of 26*
31 *carbon atoms*), which is defined as:

1

$$2 \quad \text{HG}_{26} = \text{HG}_{26} \text{ keto-ol} / (\text{HG}_{26} \text{ diol} + \text{HG}_{26} \text{ keto-ol}). \quad (1)$$

3

4 This notation, however, is somewhat counterintuitive as values of the HG_{26} -index decline
5 with increasing growth temperature. Therefore, we here used the HDI_{26} (*heterocyst diol index*
6 of 26 carbon atoms), which in contrast to the HG_{26} -index is positively correlated with
7 temperature and defined as given below. It should be pointed out though that the HG_{26} -index
8 and the HDI_{26} have the same statistical significance.

9

$$10 \quad \text{HDI}_{26} = \text{HG}_{26} \text{ diol} / (\text{HG}_{26} \text{ keto-ol} + \text{HG}_{26} \text{ diol}), \quad (2)$$

$$11 \quad \text{HDI}_{26} = 0.0224 \times \text{SWT} + 0.4381; r^2 = 0.93. \quad (3)$$

12

13 In Lake Schreventeich, HDI_{26} values ranged from 0.89 in mid-August to 0.66 in late October
14 (Fig. 4) and closely followed variations in surface water temperatures (Fig. S2 in the
15 Supplement). For example, HDI_{26} values gradually declined over the investigated time period
16 until mid-October and afterwards slightly increased again in agreement with a rise in
17 measured surface water temperature in late October. Least squares analysis of the data
18 showed that variations in HDI_{26} values are strongly linearly correlated with surface water
19 temperatures. As the HG_{28} diol and keto-ol as well as the HG_{28} triol and keto-diol showed
20 similar changes in fractional abundances compared to the HG_{26} diol and the HG_{26} keto-ol, we
21 also employed the HDI_{28} (*heterocyst diol index of 28 carbon atoms*) and the HTI_{28} (*heterocyst*
22 *triol index of 28 carbon atoms*) in order to quantitatively determine changes in HG
23 distributions with environmental parameters. Both indices were calculated as given in the
24 following equations:

25

$$26 \quad \text{HDI}_{28} = \text{HG}_{28} \text{ diol} / (\text{HG}_{28} \text{ keto-ol} + \text{HG}_{28} \text{ diol}), \quad (4)$$

$$27 \quad \text{HDI}_{28} = 0.0405 \times \text{SWT} + 0.0401; r^2 = 0.70, \quad (5)$$

28

$$29 \quad \text{HTI}_{28} = \text{HG}_{28} \text{ triol} / (\text{HG}_{28} \text{ keto-diol} + \text{HG}_{28} \text{ triol}), \quad (6)$$

1 $HTI_{28} = 0.0288 \times SWT + 0.2292; r^2 = 0.78.$ (7)

2

3 Similar to the HDI_{26} , the HDI_{28} and the HTI_{28} closely followed measured surface water
4 temperatures with absolute values of these indices gradually declining over the investigated
5 time period from 0.82 to 0.42 and from 0.81 to 0.49, respectively (Fig. 4). Least squares
6 analysis of the data demonstrates that both indices are significantly correlated with surface
7 water temperatures, although correlations are generally less strong as compared to the HDI_{26} .
8 All three HG indices, however, seem to track temperature changes in the lake's surface waters
9 in a similar fashion, albeit with slight differences in absolute values and trends between the
10 individual indices (see Fig. S2 in the Supplement). One explanation for the slight offsets
11 between the individual indices may be the contribution of heterocyst glycolipids from
12 different cyanobacterial sources. Bauersachs et al. (2009a; 2014) as well as Wörmer et al.
13 (2012) noticed that fractional abundances of heterocyst glycolipids may vary between
14 different genera of heterocystous cyanobacteria and even within heterocystous cyanobacteria
15 belonging to the same genus. Moreover, Bauersachs et al. (2014) observed that fractional
16 abundances of HG_{26} and HG_{28} diols and keto-ols changed differently in *Anabaena* CCY9613
17 and *Nostoc* CCY9926, resulting in slightly different HGI_{26} and HGI_{28} values for each of the
18 investigated species. As multiple members of heterocystous cyanobacteria (e.g., *Anabaena*
19 and *Aphanizomenon*), adapting the composition of the heterocyst cell envelope in slightly
20 different fashions, likely contributed to the total pool of HGs in Lake Schreventeich, absolute
21 values of the different HG indices may have varied depending on the amount of heterocyst
22 glycolipids contributed by each individual cyanobacterium. In this context it is interesting to
23 note that the different HG indices show a similar trend with surface water temperatures but
24 that HDI_{26} values are generally higher compared to HDI_{28} and HTI_{28} values, resulting in a
25 deviation from the 1:1 line as shown in Fig. S3. HDI_{28} and HTI_{28} values on the contrary are
26 very similar to each other and fall close to the 1:1 line, indicating that they may have the same
27 biological origin.

28 When the different HG indices are plotted against environmental parameters other than
29 surface water temperatures (Fig. 4), it is apparent that the HDI_{26} ($p < 0.001$; $r^2 = 0.64$) and the
30 HTI_{28} ($p < 0.05$; $r^2 = 0.42$) are positively correlated with decreasing oxygen concentrations
31 and that the HDI_{26} ($p < 0.05$; $r^2 = 0.35$) and the HDI_{28} ($p < 0.05$; $r^2 = 0.35$) also show a weak
32 positive correlation with pH. However, these correlations are generally less significant and

1 not as strong as observed for the correlation with surface water temperatures. It should also be
2 noted that oxygen concentrations and pH are strongly correlated with surface water
3 temperatures and that both parameters show a positive correlation with each other (Table 1).
4 Therefore, the observed correlations between the different HG indices and oxygen
5 concentrations as well as pH are likely indirect rather than indicating a statistically significant
6 relationship between the individual environmental parameters and changes in the heterocyst
7 glycolipid distribution. However, Kangatharalingham et al. (1992) reported that the heterocyst
8 cell envelope of *Anabaena flos-aquae* increased in thickness when this cyanobacterium was
9 grown under increased levels of oxygen stress and it can therefore not be excluded that
10 environmental factors other than growth temperature may affect the distribution of heterocyst
11 glycolipids in heterocystous cyanobacteria (although these authors did not analyze changes in
12 the chemical structure of the heterocyst cell envelope). Additional investigations employing
13 culture-dependent approaches and studying the effect of environmental parameters other than
14 growth temperature will be needed to elucidate whether and to which extent oxygen
15 concentrations and pH exert a control on the structural composition of the heterocyst cell
16 envelope of heterocystous cyanobacteria.

17 **4.2 Accuracy of surface water reconstructions based on HG indices**

18 The accuracy with which surface water temperatures of a given aquatic environment can be
19 reconstructed is essential for any novel lipid thermometer. Based on replicate analysis of
20 individual water column filtrates and surface sediments, the average analytical precision with
21 which the HDI₂₆ can be determined is ± 0.006 . Using the respective temperature calibration
22 (see Eq. 3), this equals a standard error in temperature estimates of $\pm 0.27^\circ\text{C}$. The
23 determination of HDI₂₈ (± 0.012) and HTI₂₈ (± 0.010) values is slightly less accurate than for
24 the HDI₂₆, which may be due to the lower abundance of HG₂₈ diols, triols, keto-ols and keto-
25 diols in the analyzed water column filtrates, with the standard error in temperature estimates
26 being $\pm 0.30^\circ\text{C}$ for the HDI₂₈ and $\pm 0.34^\circ\text{C}$ for the HTI₂₈. However, the overall analytical
27 precision in the analysis of the different HG indices is in the same order of magnitude or even
28 slightly better when compared to other well-established temperature proxies, such as the
29 TEX₈₆ and U^K₃₇, and indicates that reconstructions of surface water temperatures using the
30 HDI₂₆ and other HG indices may be achieved in a relatively high accuracy. This is also
31 suggested by analysis of the residual errors of the HG-estimated SWTs (calculated SWTs –
32 measured SWTs), which are generally $< 2^\circ\text{C}$ with a mean standard error of 0.97°C , 1.62°C

1 and 1.69°C for HDI₂₆-, HDI₂₈- and HTI₂₈-reconstructed SWTs, respectively, and without
2 following a clear trend with SWT (see Fig. S4 in the Supplement).

3 **4.3 Distribution of heterocyst glycolipids in Lake Schreventeich surface** 4 **sediments**

5 In order to determine if the heterocyst glycolipid signal observed in the water column filtrates
6 is transferred to the sedimentary realm, we also analyzed two surface sediments collected
7 from Lake Schreventeich for their HG content. Sedimentary HG distributions were indeed
8 very similar to those observed in water column filtrates with HG₂₆ diol and HG₂₆ keto-ol
9 dominating over smaller quantities of HG₂₈ triol and HG₂₈ keto-diol as well as HG₂₈ diol and
10 HG₂₈ keto-ol. It is interesting to note that the distribution of HGs in the two surface sediment
11 samples most closely resembled the one observed during the period of maximum lake
12 productivity and peak abundances of HGs in early to mid-September (Figs. 2 and 3),
13 suggesting that the preserved HGs were mainly produced during maximum activity of
14 heterocystous cyanobacteria in Lake Schreventeich. HDI₂₆ values of surface sediments from
15 Lake Schreventeich averaged 0.791±0.008. Using the temperature calibration obtained from
16 the analysis of the water column filtrates, the HDI₂₆ value translates into an average surface
17 water temperature of 15.8±0.3°C. Considering the current accuracy of the HPLC/MS method
18 for the HDI₂₆ analysis, the HDI₂₆-based temperature reconstructed for Lake Schreventeich
19 largely agrees with surface water temperatures measured from early to mid-September and
20 thus during the time period of highest productivity of heterocystous cyanobacteria. Likewise,
21 reconstructed surface water temperatures based on HDI₂₈ (0.575±0.018) and HTI₂₈
22 (0.637±0.012) values obtained from the analysis of surface sediments of Lake Schreventeich
23 and using their respective temperature calibrations are 13.1±0.4°C and 14.1±0.3°C,
24 respectively. Although slightly lower than the HDI₂₆-based SWT estimates, both values again
25 agree well with surface water temperatures measured during mid-September. Together these
26 observations suggest that the analysis of sedimentary HGs may allow reconstructing summer
27 surface water temperatures in Lake Schreventeich and possibly also other lacustrine
28 environments with sufficient export and incorporation of cyanobacterial-derived organic
29 matter into the sediment.

30 Despite the good agreement between measured and reconstructed surface water temperatures,
31 it should be pointed out that the recovered surface sediments most likely not only contained
32 HGs produced during the investigated time interval but HG distributions probably reflect a

1 time-integrated signal that covers several years. In addition, surface water temperatures of
2 Lake Schreventeich are expected to vary over the time-course of a day and the obtained
3 temperatures (though always recorded at the same time of the day) provide only a snap shot of
4 the actual temperature variance of the lake. Parts of the uncertainties in the correlation of HG
5 indices and surface water temperatures may in fact be related to the low number of diurnal
6 temperature measurements but may be improved by continuous temperature logging of the
7 lake's surface waters in future studies. As discussed above, contributions of HGs from
8 heterocystous cyanobacteria with slightly different HG distribution patterns and absolute
9 abundances of HGs may also result in the observed offsets between the HG-based SWT
10 calculations. Nonetheless, the overall good agreement of HG distributions in surface
11 sediments and water column filtrates seems to indicate that HGs in Lake Schreventeich are
12 largely produced in late summer, coinciding with blooms of heterocystous cyanobacteria, and
13 that HG-reconstructed surface water temperatures primarily reflect a summer signal in this
14 temperate lake.

15 **4.4 Geochemical implications**

16 As mentioned previously, N₂-fixing heterocystous cyanobacteria are a common component of
17 the phytoplankton community in contemporary freshwater and brackish environments of polar
18 to tropical latitudes, where they may form massive blooms during summer (Whitton, 2012).
19 Likewise, HGs seem to be widely distributed in modern freshwater and brackish
20 environments. They have been reported from surface sediments of several European and
21 African lakes including Lake Ohrid, Lake Malawi and Lake Challa (Bauersachs et al., 2010)
22 as well as in phytoplankton collected from a number of Spanish freshwater reservoirs
23 (Wörmer et al., 2012). HG distributions dominated by HG₂₆ and HG₂₈ diols have been
24 reported from core top sediments recovered from the Landsort Deep, Baltic Sea (Bauersachs
25 et al., 2010). They have also been described in several microbial mats growing along the coast
26 of the southern North Sea (Bauersachs et al., 2011; Bühring et al., 2014) and western
27 Spitsbergen (Rethemeyer et al., 2010) as well as in an Icelandic hot spring (Bauersachs et al.,
28 2013). A suite of HG₂₆ to HG₂₈ diols, triols, keto-ols and keto-diols was detected in suspended
29 particulate matter in the surface waters of 23 oligotrophic and eutrophic lakes in Minnesota
30 and Iowa, USA (Schoon, 2013), while HG₂₆ to HG₂₈ diols and keto-ols were present in
31 variable abundances and distributions in microbial mats recovered from Shark Bay, Western-
32 Australia (Bauersachs et al., unpublished data).

1 The remarkable strong linear correlations found for the distribution of HGs in water column
2 filtrates of Lake Schreventeich and surface water temperatures indicates that HG distributions,
3 in form of the HDI₂₆ and other HG indices, may be well suited to track changes in water
4 temperatures of the photic zone in freshwater environments. The generally good agreement of
5 HG indices obtained from core top sediments of Lake Schreventeich with summer surface
6 water temperatures furthermore suggests that the distribution of sedimentary HGs may also
7 record surface water temperatures of lacustrine settings over time. In addition, it may suggest
8 that no or only little selective degradation of HGs (e.g., diols vs. keto-ols) upon sinking and
9 transport through the water column as well as during the incorporation into the sediment
10 record occurred in this shallow lake system. At this point, however, it cannot be ruled out that
11 microbial reworking may bias the initially-synthesized HG signal in deeper lakes. Hence,
12 additional studies determining degradation rates of individual HGs as well as changes in the
13 overall HG distribution patterns with water depth will be necessary in order to elucidate
14 whether and to which extent the HG inventory of lakes experiences early diagenetic
15 alteration. Likewise, only limited information on the preservation potential of HGs over
16 geological time scales exists. These components have been reported from Pleistocene
17 Mediterranean sapropels as well as lacustrine deposits from the Oligocene Lake Enspel and
18 the Eocene Messel oil shale (Bauersachs et al., 2010), indicating that they may readily
19 preserve in the sediment record. However, detailed studies investigating the preservation of
20 HGs in sedimentary sequences and their stability under varying environmental conditions are
21 currently missing but will be essential to determine the robustness of HGs as lipid
22 paleothermometers.

23 It has previously been demonstrated that changes in the distribution of HGs as a function of
24 growth temperature can vary significantly between different cyanobacterial species as
25 reported for *Anabaena* CCY9613 and *Nostoc* CCY9928 (Bauersachs et al., 2014). A finding
26 that we confirmed for other nostocalean cyanobacteria such as *Aphanizomenon* sp. and
27 *Nodularia* sp. in recent culture experiments (Bauersachs et al., unpublished data). The
28 application of HDI₂₆ and other HG-based indices may thus potentially be biased in lakes that
29 are characterized by simultaneous growth of multiple species of heterocystous cyanobacteria,
30 each modifying the composition of the heterocyst cell envelope in a slightly different fashion.
31 It should also be pointed out that core top calibrations (such as those obtained from Lake
32 Schreventeich) may not be applicable to accurately determine surface water temperatures in
33 lake environments, in which the cyanobacterial community gradually changed over time.

1

2 **5 Conclusion**

3 The presence of heterocyst glycolipids in core top sediments of Lake Schreventeich, the
4 overall good agreement of HG-based temperature estimates with measured surface water
5 temperatures and the ubiquitous distribution of heterocystous cyanobacteria in modern
6 freshwater and brackish environments, suggests that the HDI_{26} and other HG-based indices
7 may hold great promise as proxies for the reconstruction of surface water temperatures in
8 modern and possibly also fossil lacustrine environments, something that is currently not
9 achieved by any other organic geochemical proxy. As heterocyst glycolipids constitute highly
10 specific biological markers for diazotrophic heterocystous cyanobacteria, they also allow a
11 direct study of the overall impact of surface water temperature changes on the cyanobacterial
12 community structure of a given lake system. However, additional analyses of HG
13 distributions in freshwater environments in combination with environmental parameters (such
14 as water temperatures, oxygen concentrations, pH, light intensities etc.) and molecular studies
15 are clearly needed to evaluate the potential use of HG-based proxies in the determination of
16 lacustrine surface water temperatures on a larger scale.

17

18 **Author contribution**

19 T.B. and L.S. designed the experiments. J.R. was involved in sample collection, the
20 determination of the physical properties of the lake's surface waters and quantification of
21 phytoplankton biomass. T. B. analyzed the water column filtrates for their HG content and
22 prepared the manuscript with contributions from all co-authors.

23

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28

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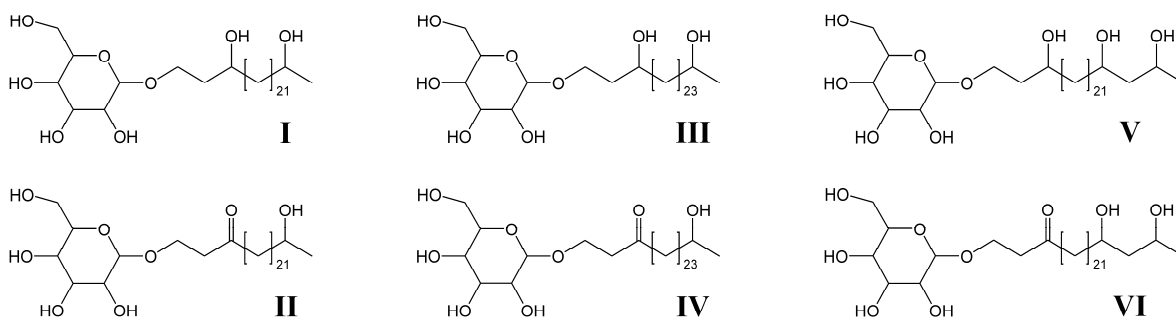
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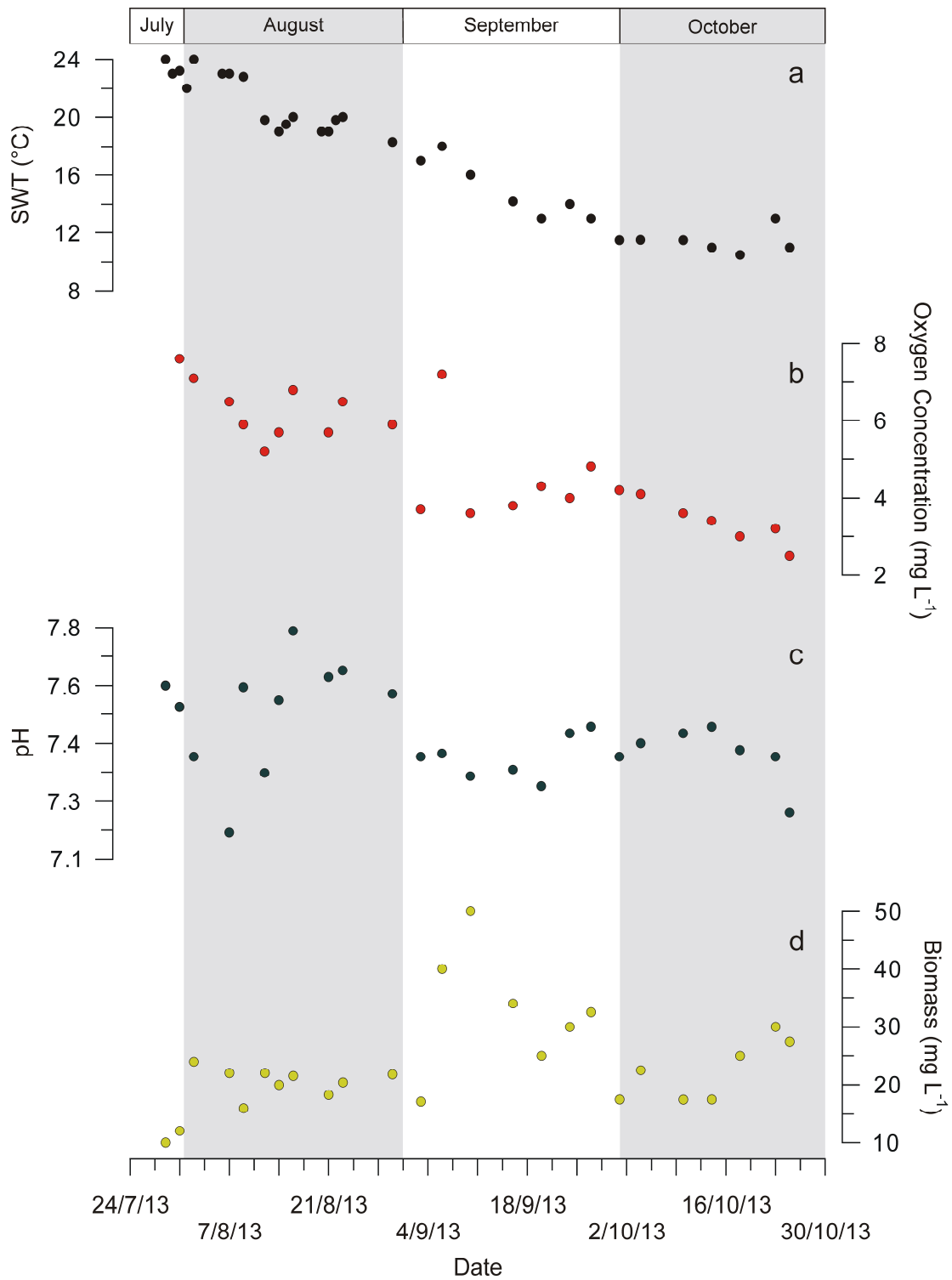
1 Table 1. Correlations of fractional abundances (F) of individual heterocyst glycolipids and
 2 heterocyst glycolipids indices with surface water temperatures (SWT), oxygen concentrations,
 3 pH and biomass. Significant correlations were, among others, observed between fractional
 4 abundances of heterocyst glycolipids and SWT as well as between the different HG indices
 5 and SWT. Note that certain environmental parameters were also positively correlated with
 6 each other. Significant correlations are indicated in bold. r = Correlation coefficient; p = p -
 7 value.

Parameter		SWT (°C)	Oxygen Con. (mg l ⁻¹)	pH	Biomass (mg l ⁻¹)
$F_{\text{HG26 diol}}$	r	0.807	0.746	0.424	0.216
	p	0.000	0.001	0.115	0.439
$F_{\text{HG26 keto-ol}}$	r	-0.954	-0.777	-0.671	0.027
	p	0.000	0.001	0.006	0.925
$F_{\text{HG28 diol}}$	r	-0.714	-0.621	0.494	-0.444
	p	0.009	0.031	0.103	0.148
$F_{\text{HG28 keto-ol}}$	r	-0.715	-0.467	0.624	-0.571
	p	0.009	0.126	0.030	0.052
$F_{\text{HG28 triol}}$	r	0.680	0.445	0.856	-0.257
	p	0.007	0.111	0.000	0.374
$F_{\text{HG28 keto-diol}}$	r	-0.288	-0.251	0.550	-0.574
	p	0.318	0.387	0.042	0.032
HDI ₂₆	r	0.962	0.803	0.591	0.070
	p	0.000	0.000	0.020	0.805
HDI ₂₈	r	0.835	0.530	-0.590	0.624
	p	0.001	0.077	0.044	0.030
HTI ₂₈	r	0.884	0.646	0.109	0.451
	p	0.000	0.013	0.711	0.105
SWT (°C)	r		0.866	0.335	-0.316
	p		0.000	0.101	0.124
Oxygen Con. (mg L ⁻¹)	r			0.430	-0.232
	p			0.036	0.275
pH	r				-0.415
	p				0.039



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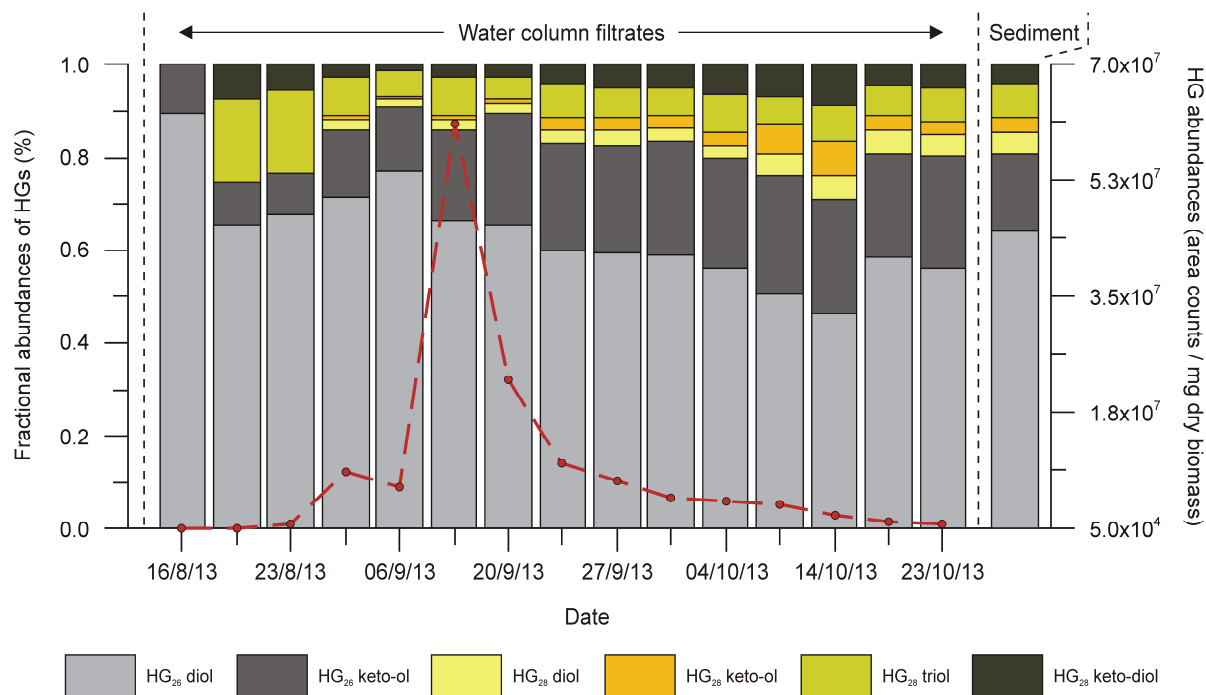
Figure 1. Structures of heterocyst glycolipids detected in water column filtrates and surface sediments of Lake Schreventeich. 1-(O-hexose)-3,25-hexacosanediol (**I**), 1-(O-hexose)-3-keto-25-hexacosanol (**II**), 1-(O-hexose)-3,27-octacosanediol (**III**), 1-(O-hexose)-3-keto-27-octacosanol (**IV**), 1-(O-hexose)-3,25,27-octacosanetriol (**V**) and 1-(O-hexose)-3-keto-25,27-octacosanediol (**VI**).



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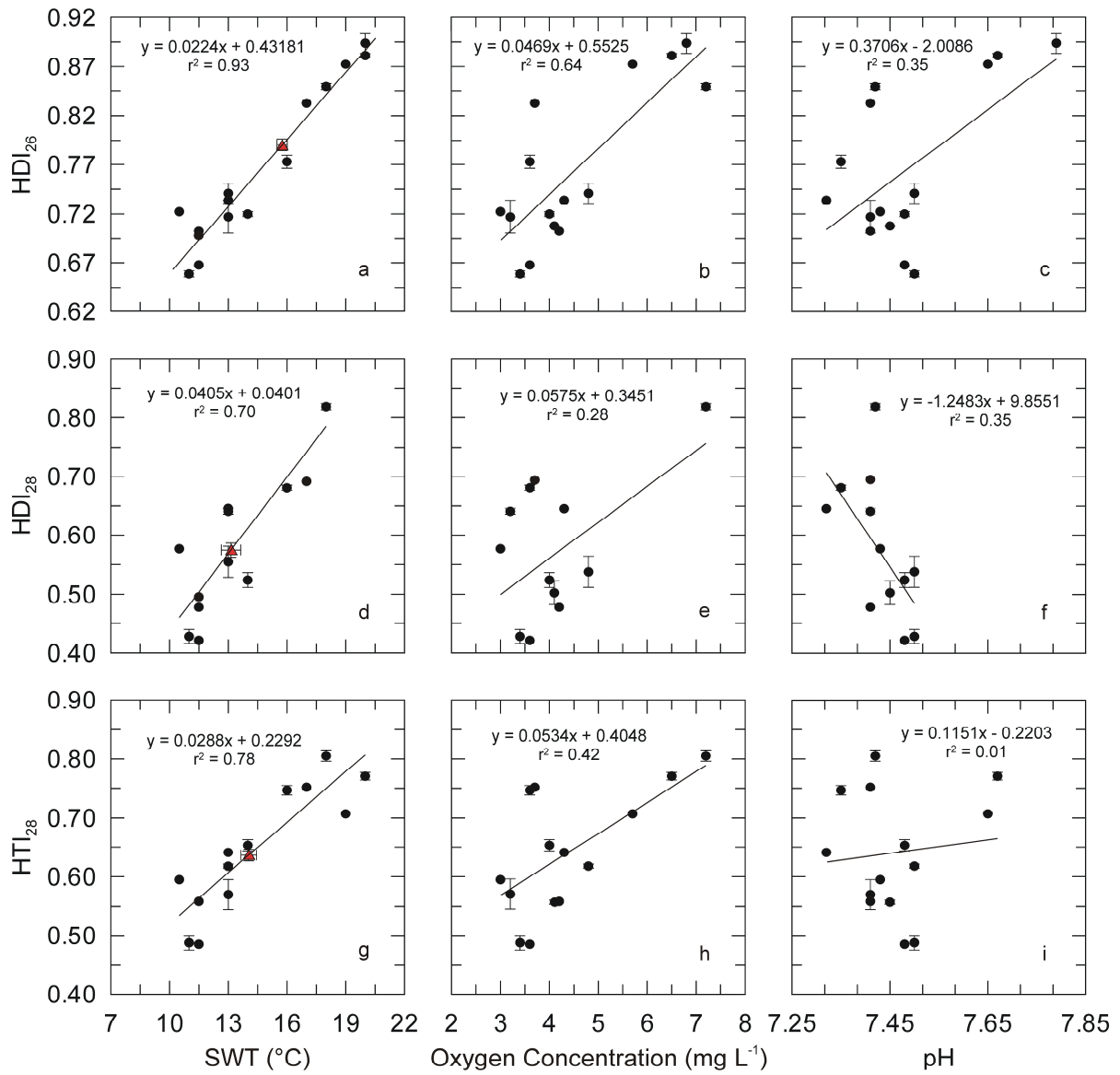
2 Figure 2. (a) Surface water temperatures (SWT), (b) oxygen concentrations, (c) pH and (d)
 3 amount of phytoplankton biomass measured in Lake Schreventeich from late July until the
 4 end of October 2013.

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Figure 3. Fractional abundances of heterocyst glycolipids (HGs) in surface waters of Lake Schreventeich. Dashed line indicates relative abundances of the sum of all heterocyst glycolipids over the investigated time interval. Note that heterocyst glycolipids were not detected in water column filtrates taken before mid-August. Fractional abundances of HGs in the sediment of Lake Schreventeich represent average values obtained from the analysis of two core top samples.



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Figure 4. Cross plots of the HDI₂₆ (a-c), HDI₂₈ (d-f) and HTI₂₈ (g-i) obtained from water column filtrates with measured surface water temperatures (SWT), oxygen concentrations and pH of Lake Schreventeich's surface waters. Red triangles represent HDI₂₆-, HDI₂₈- and HTI₂₈- reconstructed SWT obtained from the analysis of surface sediments of Lake Schreventeich.