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Functional variability of dissolved organic matter from the surface water of a productive lake

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Abstract

Functional variability of dissolved organic matter (DOM) from the surface water of Esthwaite Water (N. England), was investigated using a series of 12 standardised assays, which provide quantitative information on light absorption, fluorescence, photochemical fading, pH buffering, copper binding, benzo(a)pyrene binding, hydrophilicity and adsorption to alumina. Ten lakewater samples were collected at different times of year during 2003-2005, and DOM concentrates obtained by low-temperature rotary evaporation. Suwannee River Fulvic Acid was used as a quality control standard. For 9 of the assays, variability among DOM samples was significantly ($p<0.01$) greater than could be explained by analytical error. Seasonal trends observed for 6 of the assays could be explained by a simple mixing model in which the two end-members were DOM from the catchment (allochthonous) and DOM produced within the lake (autochthonous). The fraction of autochthonous DOM predicted by the model is significantly correlated ($p <0.01$) with chlorophyll concentration, consistent with production from phytoplankton. Autochthonous DOM is less light-absorbing, less fluorescent, more hydrophilic, and possesses fewer proton-dissociating groups, than allochthonous material.

Key words: allochthonous; autochthonous; chlorophyll a; dissolved organic matter; functions; lakes

1. Introduction

Dissolved organic matter (DOM) in natural waters participates in many important ecological and geochemical reactions (Perdue and Gjessing, 1990; Kullberg et al., 1993; Hessen and Tranvik, 1998). For example, it controls the transport and fate of heavy metals, aluminium, radionuclides and organic pollutants, initiates photoreactions, participates in particle surface and colloid chemistry, and affects ionic balance, including pH. Quantitative descriptions of these functional properties are needed for ecology, geochemistry, and to understand and predict the toxicity and fate of pollutants. The need for such descriptions is given extra impetus by the apparent sensitivity of DOM to environmental change, as shown by long-term increases (Hongve et al., 2004; Evans et al., 2005) or decreases (Schindler et al., 1996) in DOM concentration, and changes in DOM quality (Curtis, 1998; Donahue et al. 1998), attributed to climatic warming and/or declining acid deposition.

Knowledge about the functional properties of DOM has been obtained largely from laboratory experiments with isolated fractions, especially humic and fulvic acids, from different natural environments, and obtained by different methods. Inevitably the data obtained are not systematic, which makes it difficult to apply the available knowledge to field situations. Given that freshwater DOM molecular structure, composition, and size are considered to vary considerably, depending upon (i) source material (Malcolm, 1990; Curtis, 1998), (ii) differential retention during passage through soils (Kaiser et al., 2002), and (iii) modification in the freshwater system, notably by photolysis (Waiser and Robarts, 2000), it seems inevitable that functional properties will vary as well. However, at present we cannot readily relate DOM function to structure.

To address the issue of functional variability in DOM directly, Thacker et al. (2005) developed standardised assays, that can be applied to DOM isolates in order to quantify variability in the functional properties of DOM. The 11 assays, together with one additional assay, are summarised in Table 1. In each case, solutions of isolated DOM are prepared under standardised conditions, and a functional property is measured. A key feature of the approach is the use of a quality control standard (Suwannee River Fulvic Acid, SRFA) which is analysed alongside each DOM sample. The assays of optical absorbance (1, 2 and 12) characterise the effect of DOC on light penetration of surface waters, while determinations of photodecomposition (assay 4) and fluorescence (assay 3) are relevant to photochemical activity. Assays 5, 6 and 7 quantify interactions of DOM with other solutes, and are relevant to natural water chemistry and the transport and bioavailability of essential and potentially-

toxic metals and hydrophobic organic contaminants. The hydrophilicity assays (8 and 9) are relevant to aggregation, and sorption processes involving cells and mineral surfaces, while the adsorption assays (10 and 11) deal directly with mineral adsorption.

In lakes, two sources of DOM can broadly be distinguished. Allochthonous DOM (DOM_{ALL}) originates from the catchment, mainly through the decay of terrestrial plant material and subsequent leaching of partial decomposition products. Autochthonous DOM (DOM_{AUT}) is produced within the lake itself. Thomas (1997) identified three main sources of DOM_{AUT}; (i) sloppy feeding or excretion by living organisms (bacteria, phytoplankton, invertebrates and fish); (ii) bacterial degradation of dead particulate organic matter (in epilimnion, hypolimnion and sediment); (iii) abiotic polymerisation and degradation. Macrophytes may also contribute. "Autochthonous-like" DOM may be produced from DOM_{ALL}, due to in-lake chemical alterations, for example, acidification (Donahue et al. 1998) and photobleaching (Waiser and Robarts, 2000). Typically, DOM_{AUT} absorbs less UV light, is poorer in aromatic residues, and is more enriched in nitrogen than DOM_{ALL} (Tipping et al., 1988; Curtis and Adams, 1995; Curtis 1998). There are also differences in fluorescence properties, for example Donahue et al. (1998) reported that, with excitation at 370 nm, the peak emission of DOM_{ALL} was at 462 nm, whereas that of DOM_{AUT} was at 443 nm. The relative contributions of DOM_{ALL} and DOM_{AUT} in a lake depend upon hydrological factors and the biological and physico-chemical characteristics of the water body and its surrounding catchment (Thomas, 1997).

Thacker et al. (2005) observed significant differences between functional properties of DOM from a eutrophic lake (Esthwaite Water, EW) and those of DOM from three stream waters, one of which was an inflow to EW. Differences between the two EW samples were attributed to seasonal differences in the content of DOM_{AUT} (see also Tipping et al., 1988). In the present work we investigated the functional properties of DOM in the surface water of EW in more detail, and attempted to explain seasonal variability with a two end-member (DOM_{ALL} and DOM_{AUT}) mixing model. We applied the 12 assays of Table 1 to a series of samples representative of the mixed surface water of the lake, and collected at different times of year.

2. Methods

Heaney et al. (1986) provide a comprehensive description of the physics, chemistry and biology of Esthwaite Water (54° 21´N, 2° 59´W). The catchment of the lake has an area of 17.1 km^2 and receives 1800 mm of rainfall per year on average, of which 60% falls in winter (October-March). The annual mean temperature is *c.* 10 °C, with monthly averages that range from *c.* 5 °C in January to 15 °C in July. The lake is rarely covered with ice. The lake has a surface area of 1.00 km^2, mean and maximum depths of 6.4 m and 15.5 m respectively, and a mean residence time of 13 weeks. Esthwaite Water stratifies thermally in summer, and then has an anoxic hypoliminion. There is an annual plankton cycle, estimated by the concentration of the photosynthesis pigment chlorophyll a, denoted as [Chl a]. During the period of study phytoplankton was dominated by diatoms (*Asterionella formosa)* in spring, and by blue-green algae such as *Aphanizomenon* sp. and *Woronichinia* sp. in late summer (M. DeVille, pers. comm.). Typical Chl a levels range from approximately 1 $\mu g\ l^{-1}$ in winter to 60 $\mu g\ l^{-1}$ in late summer. Relevant chemical characteristics of the samples taken in the present work are given in Table 2. These data are representative of the lake at all times, except during short periods in summer when high algal productivity causes higher pH (Maberly, 1996).

Samples (50 l) were collected by wading into the small stream that is the lake outflow. The streamwater is representative of either the whole mixed lake (winter) or the epilimnion of the stratified lake (summer). A polyethylene beaker and funnel were used to transfer water to thoroughly-rinsed 10-litre polyethylene bottles. Collection took approximately 10 minutes, and was performed between 9.00 and 12.00 hours. Samples were returned to the laboratory within one hour, and stored cold and dark during processing.

The method used to isolate the DOM is described in detail by Thacker et al. (2005) and involved concentrating the filtered (GF/F Millipore, nominal pore size 0.7 μm) sample to approximately 500 cm^3, using a high capacity, low pressure, low temperature (20 ºC), rotary-evaporator (Buchi Rotavapor R-220). The sample was then passed through a column of Amberlite IR-120 (in the sodium form) to exchange major cations, and filtered through Whatman GF/F and Millipore 0.22μm filters. In two cases (EW4 and EW10), a second isolation was carried out, in which the final volume was 1000 cm^3 instead of 500 cm^3.

The raw water samples and concentrates were analysed within one week for pH (Radiometer GK2401C combination glass electrode), DOC (TOC-VCPN/CPN analyzer, Shimadzu, Kyoto, Japan), absorbance at 340 nm (Hitachi U-2000 Spectrophotometer), and

conductivity (Jenway 4510 meter). Stored samples were analysed later for major cations (ICP-OES, Perkin Elmer Optima 4300 DV). Raw water samples were also analysed for alkalinity (Gran titration), major anions (Dionex DX100) and Chl a by extraction with boiling methanol (Talling, 1974).

The eleven standardised assays, previously tested and described in detail by Thacker et al. (2005), together with one additional optical absorbance assay (Table 1), were applied to the concentrates. For each assay, the DOM was present at a fixed concentration (10 to 100 mg DOC l^{-1} depending upon the measurement), in a solution of defined chemical composition, so that differences in the measured quantity reflected differences in the DOM, and not, for example, in the composition of the raw water sample. A quality control standard, reference Suwannee River Fulvic Acid (SRFA) purchased from the International Humic Substances Society, was analysed simultaneously with the samples to characterise assay reproducibility.

The extra assay of optical absorbance (at 254 nm) was added to increase the comparability of our results with other published data (e.g. Chin et al., 1994). However, the same numbering system has been maintained for the assays as in the previous work, with the optical absorbance assay at 254 nm numbered as assay 12 (Table 1).

Two modifications were made to the assays described in Thacker et al. (2005). First, an extra quality control standard was formulated for the hydrophilic assay. This was done because the SRFA quality standard is isolated on the basis of its hydrophobic character, i.e. by adsorption onto DAX-8 resin in acid solution, and therefore has a low content of hydrophilic material. To obtain similar results for both standard and samples, to aid statistical analysis, a new quality control standard was prepared by mixing 15 mg DOC l^{-1} of SRFA with 5 mg DOC l^{-1} of sodium acetate, to provide a hydrophilic component. Second, the assay output for buffer capacity assay was altered to the number of acid groups titrated between pH 4 and 8, due to the possibility of silicate interference. In Thacker et al. (2005), the number of acid groups was titrated between pH 4 and 9. The results in Thacker et al. (2005) were reanalysed and it was found that variability among the DOM samples is still significantly ($p<0.01$) greater than can be explained by analytical error, i.e. there is no change to the overall conclusion from the previous work.

It was also found by Thacker et al. (2005) that benzo(a)pyrene binding results for the DOM samples did not vary significantly. To check if this phenomenon could be an artefact of the method, additional measurements were made on a commercially-available humic acid (Aldrich Chemical Company), which has a greater affinity for hydrophobic xenobiotics than

182 does natural DOM (Kukkonen, 1991). Aldrich humic acid gave a log K_p for benzo(a)pyrene
183 binding of 5.11, 0.57 log units higher than the SRFA quality control and 0.49 log units higher
184 than the DOM samples, proving that the lack of variation shown by natural water samples was
185 not an artefact of the method.

3. Results

3.1. Esthwaite Water

Raw water samples collected from EW during 2003, 2004 and 2005, all have similar chemistries (Table 2). From fortnightly monitoring, Tipping et al. (1988) reported [DOC] in EW to remain relatively constant throughout winter (November to March) with an average of 2.0 mg l^{-1} while during summer (May to September) it was higher, with an average of 3.7 mg l^{-1}, and the more limited number of observations of the present work are consistent with this pattern. The increase in [DOC] during summer was attributed to within-lake production of DOC as a result of plankton growth and excretion and/or decomposition.

Phytoplankton biomass (μg Chl a l^{-1}) in EW is highly variable seasonally. Determinations of Chl a were made fortnightly during 2003, 2004 and 2005 (M. DeVille, pers. comm.) and the data show spring and summer maxima. Values of [Chl a] determined on samples collected for DOM assays are also shown in Table 2.

3.2. Isolation and concentration of DOM

The isolation method gave an average DOC yield of 77% (ranging from 70% to 89%). Thacker et al., (2005) concluded that the low recovery is caused by precipitation of calcium carbonate forming during the last stages of concentration and removing some DOM by adsorption or co-precipitation. A strong correlation (r = -0.92) was found between E_{340} values of raw water samples and % recovery. Furthermore, samples with the highest raw water E_{340} values underwent appreciable decreases in E_{340} on concentration (Fig. 1). These results show that DOM lost during the isolation method is from the most strongly light-absorbing fraction. Therefore, the magnitude of the loss of DOM depends on (i) the proportion of the strongly light-absorbing fraction in the raw water sample, comprising the larger molecules with a higher aromatic and hydrophobic character, and (ii) sufficiently high concentrations of Ca^{2+} and CO_3^{2-} for precipitation to occur during the concentration process.

To investigate the effect of DOM losses on measured functional properties, in two cases (EW4 and EW10), a second sample was processed, concentrated to 1000 cm^3 instead of the usual 500 cm^3. By reducing the concentration factor, improved yields were obtained, from 72% to 87% for EW4 and from 78% to 84% for EW10. The less-concentrated samples are referred to as EW4A and EW10A. Assay results for the four concentrates are shown in Table 3.

220 *3.3. Variability in DOM functional properties*

221 Figure 2 shows that for most of the assays good reproducibility was obtained for the
222 quality control standard, SRFA, with relative standard deviations (RSD) of less than 5%. The
223 fluorescence assay gave an RSD of 6.5%, while an RSD of 14.8% was obtained for the assay
224 of hydrophilicity monitored by optical absorption. Results from the quality control standard
225 were used to apply the one-tailed *F*-test (Snedecor and Cochran, 1967), to assess variability in
226 functional properties of the DOM samples (Thacker et al., 2005). For 9 assays variation
227 among EW DOM samples was significantly greater ($p < 0.01$) than can be explained by
228 analytical error i.e. by comparison with results for the SRFA standard, but no statistically
229 significant variations were found for the assays of benzo(a)pyrene binding, copper binding
230 and hydrophilicity monitored by optical absorption.

231 Several functional properties (all three extinction coefficients, fluorescence, buffer
232 capacity and hydrophilicity monitored by DOC) show systematic seasonal variations, with a
233 maximum or minimum during the summer months. We therefore attempted analysis of the
234 results with a two-member mixing model, hypothesising that seasonal variability can be
235 accounted for in terms of mixtures of DOM_{AUT} and DOM_{ALL}, the functional properties of
236 DOM_{AUT} and DOM_{ALL} being assumed constant. Therefore, a given functional property, F, of
237 DOM in EW will depend on the proportions of DOM_{AUT} and DOM_{ALL}, and can be expressed
238 as

239 $$F = F_{AUT} X_{AUT} + F_{ALL} X_{ALL} \quad (1)$$

240 where F_{AUT} and F_{ALL} are values of the functional properties of the autochthonous and
241 allochthonous end-members respectively, and X_{AUT} and X_{ALL} are the fractions of those end
242 members. Since the sum of X_{AUT} and X_{ALL} must be unity, equation (1) can be written

243 $$F = F_{AUT} X_{AUT} + F_{ALL} (1 - X_{AUT}) \quad (2)$$

244 Since there are 12 assays, each applied to 10 samples, there are 120 versions of
245 equation (2). Therefore the total number of parameters to be found is 34, comprising 12
246 values each of F_{AUT} and F_{ALL}, and 10 values of X_{AUT}. Rather than using the entire data set to
247 extract parameter values, we initially confined the analysis to results for E_{254}, E_{280} and E_{340}.
248 Extinction coefficients were chosen firstly because additivity would clearly be expected on
249 mixing the two end-members, and secondly because the measurements are highly precise
250 (quality control RSD <0.5%). The 'Solver' facility of Microsoft 'Excel' was used to find
251 parameters by least-squares minimisation of the sum of squared residuals between observed
252 and predicted functional assay results.

253 The mixing model worked well, explaining 99.7% of the variance in the extinction
254 coefficients. Moreover the derived values of X_{AUT}, from 0.17 to 0.88, indicate that the
255 sampling programme produced an adequate range of mixtures of DOM_{ALL} and DOM_{AUT}. The
256 top three panels of Fig. 3 show observed values of E_{254}, E_{280} and E_{340} plotted against derived
257 values of X_{AUT}. The other panels of Fig. 3 show results for the remaining assays plotted
258 against X_{AUT}, together with the results of regression analysis. In three cases, $F_{DOC/325/450}$,
259 $Hyphil_{DOC}$%, and $Ac_{4\text{-}8}$, the functional property shows a significant ($p < 0.01$) dependence on
260 X_{AUT}. Table 4 shows F values for each assay, for the two end-members.
261
262
263

4. Discussion.

4.1 Isolation of DOM

The method to obtain DOM samples for the assay work is a compromise between full isolation, with removal of all solutes except DOM, and a mild method that produces a high yield (Thacker et al., 2005). However, the final concentrates obtained from the EW samples with higher E_{340} values were depleted in the highly coloured aromatic fraction of DOM (Section 3.1, Fig. 1). Because DOM_{ALL} has higher aromaticity, hydrophobic character and UV absorbance than DOM_{AUT} (see Table 3), isolation losses may have selectively affected the DOM_{ALL} end-member in the final concentrate. The results in Table 4 for samples EW4 (lower yield) and EW4A (higher yield) confirm this to some extent, in that EW4A gave somewhat higher values of E_{254}, E_{280}, E_{340}, $Ac_{4\text{-}8}$, Ads_{DOC}% and log K_P, and lower values of $F_{DOC/325/450}$, $Hyphil_{DOC}$% and $Hyphil_{A340}$%. However, the differences are small, and they are not reproduced by samples EW10 and EW10A. Therefore, isolation losses of DOM do not seem to have had a major selective effect on functional properties.

4.2 Variability in DOM functional properties

The successful application of the mixing model (Fig. 3, Table 4) permits the distinction of three categories of DOM functional property (Table 5). Category A comprises functional properties that vary significantly both among DOM samples and also with X_{AUT}. For the six functional properties in this category, some (in five cases, most) of the observed variability can be attributed to variations in X_{AUT}, and co-variations in X_{ALL}. As the fraction of DOM_{AUT} in EW increases, the DOM becomes less light-absorbing and less fluorescent. These results are consistent with the findings of Donahue et al. (1998) and Waiser and Robarts (2004). In addition, the present data show that DOM_{AUT} is more hydrophilic, and possesses fewer acid-dissociating groups than DOM_{ALL}. Five of the six functional properties in this category were also found to vary among the samples studied in previous work (Thacker et al., 2005); the E_{254} was not measured previously.

Category B comprises three functional properties that vary significantly among DOM samples, but do not vary with X_{AUT}. Two of the three, Ads_{DOC}% and Ads_{340nm}%, also varied amongst the samples studied by Thacker et al. (2005). The consistent variability of these two related properties is evidently due to factors other than those that control variability within category A. The photochemical fading results for EW differ from those of the other assays, by displaying a step-change between June and July, thereby giving rise to a bimodal pattern

when plotted against X_{AUT}, and significant variability. We have no explanation for this phenomenon at present. In the work of Thacker et al. (2005), significant variability in A_{340} loss% was not found.

Category C comprises three functional properties that do not vary significantly among the DOM samples, neither do they vary with X_{AUT}. Thacker et al. (2005) also found that neither copper nor benzo(a)pyrene binding varied amongst surface water samples, but they did find significant variability in hydrophilicity as measured by optical absorbance.

4.3. Sources of lakewater DOM

The mixing model permits estimation of the functional properties of the two postulated DOM end-members in Esthwaite Water, even though neither can be isolated and characterised in a "pure" state. Table 4 compares the derived properties of DOM_{ALL} with those determined by Thacker et al. (2005) for DOM samples from Esthwaite Hall Beck, a stream flowing into EW. The results are very similar for five of the six functional assays, E_{254}, E_{280}, E_{340}, $F_{DOC/325/450}$ and $Hyphil_{DOC}$. Agreement is less good for Ac_{4-8} but the result for DOM_{ALL} is much closer to the value for Esthwaite Hall Beck than is the value for DOM_{AUT}. Therefore, it can be concluded that DOM_{ALL} has functional properties consistent with those of DOM entering the lake from its catchment, which is a basic assumption of the mixing model.

A number of studies (Søndergaard et al., 2000; Jørgensen, 1986; Norrman et al., 1995), have implicated phytoplankton in the release of DOM_{AUT}. We therefore regressed X_{AUT} against [Chl a], as a measure of phytoplankton biomass, and found a significant relationship ($R^2 = 0.71$, $p<0.01$). Fig. 4 illustrates how the values of X_{AUT} follow the seasonal pattern of [Chl a] in EW. In winter, X_{AUT} tends to be low, whereas it is high in summer. The sample collected in July 2004 during the period of highest algal biomass, corresponds to the highest value of X_{AUT} (0.88) predicted by the model. The idea that phytoplankton are the main source of DOM_{AUT} is supported by the results in Table 4 which show that values of E_{254} and E_{280} derived for DOM_{AUT} are similar to those reported for DOM from Lake Fryxell (Chin et al., 1994; Weishaar et al., 2003). Lake Fryxell is a permanently ice-covered lake in Antarctica, in which DOM is derived mainly from benthic and planktonic microbial populations, with essentially no input of organic material from its surrounding watershed (Aiken et al., 1996).

Another possible source of DOM_{AUT} is the *in situ* degradation and transformation of DOM_{ALL} by photolysis and bacterial assimilation. Curtis and Schindler (1997) reported significant losses of both DOC and colour in Canadian lakes, with half-times of 166 and 122

d respectively; during this processing, the characteristics of the DOC would probably move towards those of DOM_{ALL}. The average residence time of water in EW is 90 days (Heaney et al,. 1986), and values for the summer months tend to be longer. Therefore degradation of DOM_{ALL} might well occur and contribute to DOM_{AUT}. However, the fact that concentrations of DOC increase during the summer (see Section 3.1) strongly suggests an internal source, and so conversion of DOM_{ALL} cannot be considered the major source of DOM_{AUT}.

4.4 Implications of the results

This study and the previous work by Thacker et al. (2005) demonstrate statistically significant variability in a number of the functional properties of DOM from surface freshwaters. The results should contribute generally to the understanding of the sources and impacts of DOM in freshwaters, and more specifically to the quantitative description of freshwater systems, through predictive modelling, for example in estimating the chemical speciation of metals (Tipping, 2002), and their toxicity (Di Toro et al., 2000). The extensive data from laboratory experiments with isolated natural organic matter (mostly fulvic and humic acids) constitute a valuable resource for modelling, but average DOM properties from such studies may not be sufficient. Although it appears from Table 5 that results for SRFA would be satisfactory to predict the interactions of EW DOM with copper and benzo(a)pyrene, and its adsorption to mineral surfaces, they would overestimate the absorption of light, especially in surface waters dominated by DOM_{AUT}, and also buffering capacity, fluorescence, and hydrophobicity (see also Section 2). Thus, in principle, more precise predictions would result if DOM variability, between and within waters, were taken into account. However, ecosystem modelling inevitably involves approximation, either because of lack of input data, or incomplete process characterisation, and uncertainty arising from variability in DOM properties may be overshadowed by greater uncertainties in other factors. To understand more fully the implications of the variability demonstrated by our results, they need to be incorporated into different ecosystem models, and sensitivity analyses conducted.

5. Conclusions

1. The isolation method gave yields of 70 - 89%, with an average of 77%. The final concentrate had less absorbance per g of DOC than the raw water sample, due to preferential loss of highly coloured material during isolation.
2. For nine of the twelve assays, variability among DOM samples is significantly ($p<0.01$) greater than can be explained by analytical error, i.e. by comparison with results from the SRFA quality control standard. The three exceptions are copper binding, benzo(a)pyrene binding and hydrophilicity monitored by optical absorbance.
3. Six of the twelve functional properties of DOM in EW could be modelled in terms of mixtures of DOM from the catchment (allochthonous) and DOM produced within the lake (autochthonous).
4. Of the two DOM types, autochthonous DOM is less light-absorbing, less fluorescent, more hydrophilic, and possesses fewer proton-dissociating groups.
5. The derived properties of allochthonous DOM are similar to those of DOM in catchment streamwater. Autochthonous DOM is mainly derived from phytoplankton.

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385 **References**

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387 Aiken, G., Mknight, D., Harnish, R. and Wershaw, R., 1996. Geochemistry of aquatic humic
388 substances in the Lake Fryxell Basin, Antarctica. Biogeochem. 34 (3), 157 – 188.
389 Chin, Y-P., Aiken, G. and O'Loughlin, E., 1994. Molecular weight, polydispersity, and
390 spectroscopy properties of aquatic humic substances. Environ. Sci. Technol. 28 (11),
391 1853-1858.
392 Chin, Y.P., Aiken, G. and Danielsen, K.M., 1997. Binding of pyrene to aquatic and
393 commercial humic substances: the role of molecular weight and armaticity. Environ.
394 Sci. Technol. 31 (6), 1630-1635.
395 Curtis, P.J., 1998. Climatic and Hydrologic Control of DOM Concentration and Quality in
396 Lakes. In: Hessen, D.O. and Tranvik, L. (Eds.): Aquatic Humic Substances; Ecology
397 and Biogeochemistry. Springer-Verlag, Heidelberg pp. 93-105.
398 Curtis, P.J. and Adams., 1995. Dissolved organic matter quantity and quality from freshwater
399 and saltwater lakes in east-central Alberta. Biogeochem. 30 (1), 59-76.
400 Curtis, P.J. and Schindler, D.W., 1997. Hydrologic control of dissolved organic matter in low-
401 order Precambrian Shield lakes. Biogeochem. 36 (1), 125-138.
402 Di Toro, D.M., Allen, H.E., Bergman, H.L., Meyer, J.S., Paquin, P.R and Santore, R.C., 2001.
403 Biotic ligand model of the acute toxicity of metals. 1. Technical basis
404 Environ. Toxicol. Chem. 20(10), 2383-2396.
405 Donahue, W.F., Schindler, D.W., Page, S.J. and Stainton M.P., 1998. Acid-induced changes
406 in DOC quality in a experimental whole-lake manipulation. Environ. Sci. Technol. 32
407 (19), 2954-2960.
408 Evans, C.D., Monteith, D.T. and Cooper, D.M., 2005. Long-term increases in surface water
409 dissolved organic carbon: Observations, possible causes and environmental impacts.
410 Environ. Pollut. 137 (1), 55-71.
411 Heaney, S.I., Smyly, W.J.P. and Talling, J.F., 1986. Interactions of physical chemical and
412 biological processes in depth and time within a productive English lake during
413 summer stratification. Intl. Rev. Ges. Hydrobiol. 71 (4), 441-494.
414 Hessen, D.O. and Tranvik, L.J., 1998. Aquatic Humic Substances. Springer, Berlin.
415 Hongve, D., Riise, G. and Kristiansen, J.F., 2004. Increased colour and organic acid
416 concentrations in Norwegian forest lakes and drinking water – a result of increased
417 precipitation? Aquat. Sci. 66 (2), 231 – 238.

418 Jørgensen, N.O.G., 1986. Dissolved organic carbon (DOC) in lakes. Carbon Dynamics in
419 Eutrophic Temperate Lakes (Eds B. Riemann and M. Søndergaard), pp. 5-25. Elsevier
420 Science Publishers, Amsterdam. Pp. 5-25.
421 Kaiser, K., Guggenberger, G., Haumaier, L. and Zech, W., 2002. The composition of
422 dissolved organic matter in forest soil solutions: changes induced by seasons and
423 passage through the mineral soil. Org. Geochem. 33 (3), 307-318.
424 Kukkonen, J., 1991. Effects of dissolved organic material in fresh waters on the binding and
425 bioavailability of organic pollutants. PhD thesis summary. University of Joensuu,
426 Publs. Sci. No. 22.
427 Kullberg, A., Bishop, K .H., Hargeby, A., Jansson, M. and Petersen, R.C., 1993. The
428 ecological significance of dissolved organic carbon in acidified waters. Ambio 22 (5),
429 331-337.
430 Maberly, S.C., 1996. Diel, episodic and seasonal changes in pH and concentrations of
431 inorganic carbon in a productive lake. Freshwat. Biol. 35(3), 579-598.
432 Malcolm, R.L., 1990. The uniqueness of humic substances in each of soil, stream and marine
433 environments. Anal. Chim. Acta 232(1), 19-30
434 Norrman, B., Zweifel, U.L., Hopkinson and C. S. Jr., Fry, B. 1995. Production and utilization
435 of dissolved organic carbon during an experimental diatom bloom. Limnol. Oceanogr.
436 40 (5), 898-907.
437 Perdue, E.M. and Gjessing, E.T., 1990. Organic Acids in Aquatic Ecosystems. Wiley, New
438 York.
439 Schindler, D.W., Bayley, S.E., Parker, B.R., Beaty, K.G., Cruikshank, D.R, Fee., E.J.,
440 Schindler, E.U. and Stainton, M.P. 1996. The effects of climatic warming on the
441 properties of boreal lakes and streams at the Experimental Lake Area, Northwestern
442 Ontario. Limnol. Oceanogr. 41 (5), 1004-1017.
443 Snedecor, G.W. and Cochran, W.G., 1967. Statistical Methods, sixth ed. Iowa State
444 University Press, Ames.
445 Søndergaard, M., Borch, N.H. and Riemann, B., 2000. Dynamics of biodegradable DOC
446 produced by freshwater plankton communities. Aquat. Microb. Ecol. 23 (1), 73-83.
447 Talling, J. F. 1974. Photosynthetic pigments: general outline of spectrophotometric methods:
448 specific procedures. In: A manual for measuring primary production in aquatic
449 environments (Ed. R.A. Vollenweider), pp.22-26. Blackwell Scientific publications,
450 Oxford.

Thomas, J.D. 1997. The role of dissolved organic matter, particularly free amino acids and humic substances, in freshwater ecosystems. Freshwat. Biol. 38 (1), 1-36.

Thacker, S.A., Tipping, E., Baker, A. and Gondar, D., 2005. Development and application of functional assays for freshwater dissolved organic matter. Water Res. 39 (18), 4559-4573.

Tipping, E., 2002. Cation-Binding by Humic Substances. Cambridge University Press, Cambridge.

Tipping, E., Hilton, J. and James, B., 1988. Dissolved organic matter in Cumbrian lakes and streams. Freshwat. Biol. 19 (3), 371-378.

Waiser, M.J. and Robarts, R.D., 2000. Changes in composition and reactivity of allochthonous dissolved organic matter in a prairie saline lake. Limnol. Oceanogr. 45 (4), 763-774.

Waiser, M.J. and Robarts, R.D., 2004. Photodegradation of DOC in a shallow prairie wetland: evidence from seasonal changes in DOC optical properties and chemical characteristics. Biogeochem. 69 (2), 263-284.

Weishaar, J.L., Aiken, G.R., Bergamaschi, B.A., Fram, M.S., Fujii, R. and Mopper, K., 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environ. Sci. Technol. 37 (20), 4702-4708.

470 Table 1. Number and name of each assay, the nature of the assay result, and the abbreviated designation.

471

Assay no.	Assay	Assay result	Abbreviation
1	Optical absorbance 280 nm	Extinction coefficient[a] at 280nm (l gC^{-1} cm^{-1})	E_{280}
2	Optical absorbance 340 nm	Extinction coefficient[a] at 340nm (l gC^{-1} cm^{-1})	E_{340}
3	Fluorescence (325/450)	Peak intensity with excitation at 325nm and emission at 450nm, per mg DOC l^{-1}	$F_{DOC/325/450}$
4	Photochemical fading	% loss in DOM absorbance at 340 nm	A_{340} loss%
5	Buffering capacity	Acid groups titrated between pH 4 and 8 (meq/g C)	Ac_{4-8}
6	Copper binding	Conditional stability constant (l gC^{-1})	log K_c
7	Benzo(a)pyrene binding	Partition coefficient (cm^3 g C^{-1})	log K_p
8	Hydrophilicity (DOC)	% of DOC not adsorbed DAX-8 resin at pH 2	$Hyphil_{DOC}$%
9	Hydrophilicity (absorbance)	% of DOM absorbance (340 nm) not adsorbed by DAX-8 resin at pH 2	$Hyphil_{A340}$%
10	Alumina adsorption (DOC)	% of DOC adsorbed at pH 4	Ads_{DOC}%
11	Alumina adsorption (absorbance)	% of DOM absorbance (340nm) adsorbed at pH 4	Ads_{A340}%
12[b]	Optical absorbance 254 nm	Extinction coefficient[a] at 254nm (l gC^{-1} cm^{-1})	E_{254}

472

473 [a] Extinction coefficient; ratio of optical absorbance per cm to DOC concentration in g l^{-1}.

474 [b] Assay 12 is a new assay, in addition to the eleven assays described in Thacker et al. (2005).

475 Table 2. Chemical compositions of raw samples from Esthwaite Water.

476

Sample code	Sampling date	pH	Cond[a] μS cm^{-1}	DOC mg l^{-1}	Alk[a] mg l^{-1}	Na mg l^{-1}	Mg mg l^{-1}	Ca mg l^{1}	K mg l^{-1}	E_{340}[b] l gC^{-1} cm^{-1}	Chl a μg l^{-1}
EW1	09/10/03	7.38	119	3.9	31.2	7.21	1.5	12.1	nd[a]	7.4	14.0
EW2	27/07/04	7.87	106	3.7	24.3	6.68	1.4	11.2	0.86	5.1	52.9
EW3	17/01/05	7.50	104	2.9	20.6	6.8	1.3	9.32	1.03	9.9	2.8
EW4	21/02/05	7.64	116	2.8	22.0	7.28	1.4	10.9	1.06	8.7	1.8
EW5	20/04/05	7.63	127	2.6	24.0	7.12	1.4	10.7	0.96	12.5	9.6
EW6	18/05/05	8.00	120	3.3	27.5	7.12	1.5	11.7	1.02	10.3	23.7
EW7	16/06/05	7.59	113	3.4	26.3	6.94	1.5	11.6	0.94	7.6	8.1
EW8	20/07/05	7.87	120	3.4	24.9	7.25	1.5	11.3	0.93	8.2	15.4
EW9	23/08/05	7.82	117	3.6	27.0	7.26	1.5	11.6	0.90	5.6	26.0
EW10	13/09/05	7.84	128	2.8	26.3	7.12	1.5	11.6	0.91	8.8	21.8

477

478 [a] Cond = conductivity; Alk = alkalinity; nd = not determined

479 Table 3. Assay results for DOM samples concentrated to different extents, and therefore
480 giving different recoveries.

481
482

	EW4	EW4A	EW10	EW10A
Recovery %	72	87	78	84
E_{254}	28.3	33.2	30.6	28.7
E_{280}	21.2	25.1	22.2	20.8
E_{340}	8.0	10.0	7.1	6.6
log K_C	3.50	3.08	4.27	4.14
$Ac_{4\text{-}8}$	5.00	5.45	5.55	5.31
$F_{DOC/325/450}$	18.9	17.3	18.2	17.2
$Hyphil_{DOC}$%	37.9	37.0	45.5	45.2
$Hyphil_{A340}$%	22.7	18.3	23.7	22.9
A_{340} loss %	19.6	22.1	45.2	49.9
Ads_{DOC}%	44.9	48.7	37.1	38.6
Ads_{A340}%	72.7	75.5	59.7	61.6
log K_p	4.22	4.62	4.18	4.22

483

484 Table 4. Functional properties of DOM_{ALL} and DOM_{AUT} derived from the mixing model,
485 mean assay results for two DOM samples from Esthwaite Hall Beck (Thacker et al., 2005),
486 and SRFA, and extinction coefficients for DOM from Lake Fryxell (Weishaar et al., 2003;
487 Chin et al., 1994).
488

	ALL	EHB	AUT	L. Fryxell	SRFA
E_{254}	34.8	36.9	21.8	18.0	42.4
E_{280}	27.1	28.3	14.6	12.5	31.5
E_{340}	10.5	12.4	4.2		13.5
log K_C	3.67	4.30	4.02		3.98
Ac_{4-8}	4.22	5.31	2.71		5.42
$F_{DOC/325/450}$	21.7	18.7	9.8		15.8
$Hyphil_{DOC}$%	32.9	32.8	54.3		12.6
$Hyphil_{A340}$%	21.0	19.1	22.0		8.1
A_{340} loss %	14.9	31.1	34.1		39.6
Ads_{DOC} %	41.8	59.8	48.7		59.1
Ads_{A340} %	65.4	77.4	57.0		75.8
log K_p	4.42	4.50	4.39		4.51

489

490 Table 5. Significance of variability in functional properties. The columns headed SW and
491 EW refer to comparisons of assay results with the quality control standard for 8 surface waters
492 (SW; Thacker et al., 2005) and EW (present work). The final column refers to variations of
493 assay results with X_{AUT} values derived from the mixing model (cf. Fig. 4). S, NS = significant
494 or not significant at the 1% level.
495

Category	Assay	EW	X_{AUT}	SW
A	Optical absorbance 280 nm	S	S	S
	Optical absorbance 340 nm	S	S	S
	Fluorescence (325/450)	S	S	S
	Buffering capacity	S	S	S
	Hydrophilicity (DOC)	S	S	S
	Optical absorbance 254 nm	S	S	not used
B	Photochemical fading	S	NS	NS
	Alumina adsorption (DOC)	S	NS	S
	Alumina adsorption (absorbance)	S	NS	S
C	Copper binding	NS	NS	NS
	Benzo(a)pyrene binding	NS	NS	NS
	Hydrophilicity (absorbance)	NS	NS	S

496

497 **Figure captions**

498

499 Fig. 1. Extinction coefficients at 340 nm of raw water samples and their concentrates,
500 following isolation. The line represents a 1:1 relationship.

501

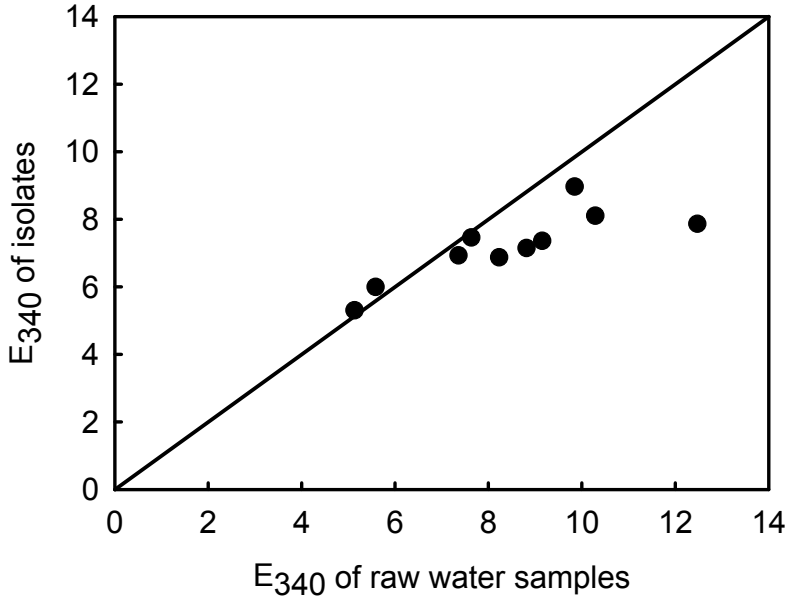
502 Fig. 2. Assay results for DOM samples from Esthwaite Water (symbols) and for the quality
503 control standard (shaded areas). Units for the y-axes are given in Table 2.

504

505 Fig. 3 Plots of functional assay results against X_{AUT}, the fraction of autochthonous DOM,
506 derived from the mixing model. Units for the y-axes are given in Table 2. The extinction
507 coefficients at 254, 280 and 340 nm (top three panels) were used to fit the model and derive
508 X_{AUT}. The remaining panels show regressions of assay results against X_{AUT}. If $R^2 > 0.40$,
509 then $p < 0.05$; if $R^2 > 0.59$, then $p < 0.01$.

510

511 Fig. 4 Seasonal variations in chlorophyll a and X_{AUT}. In the upper panel, dashed lines show
512 the range of [Chl a] for 2003 - 2005, and points are values determined on samples taken for
513 DOM isolation.


14
12
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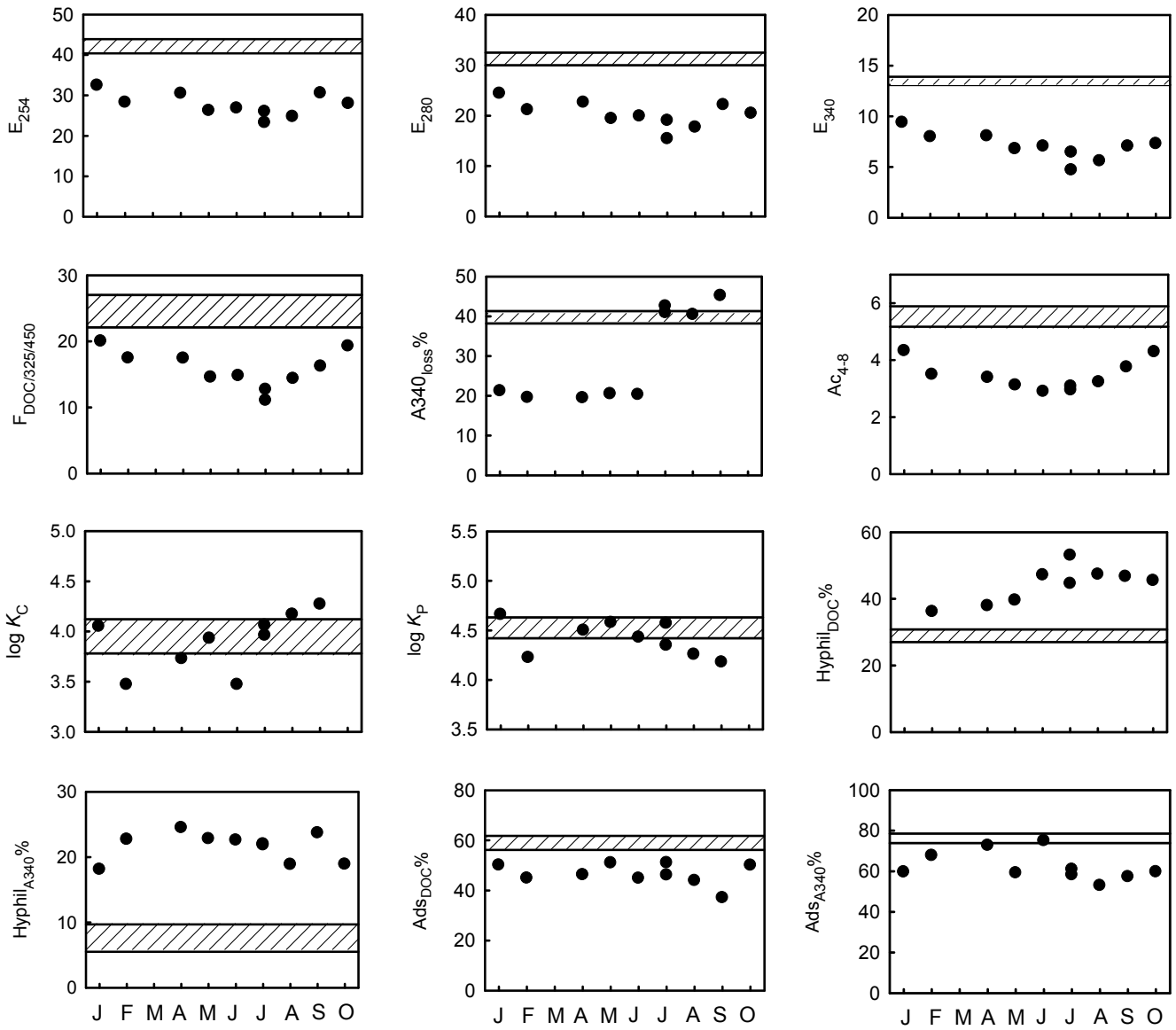
E340 of isolates
E340 of raw water samples

Fig. 1

514

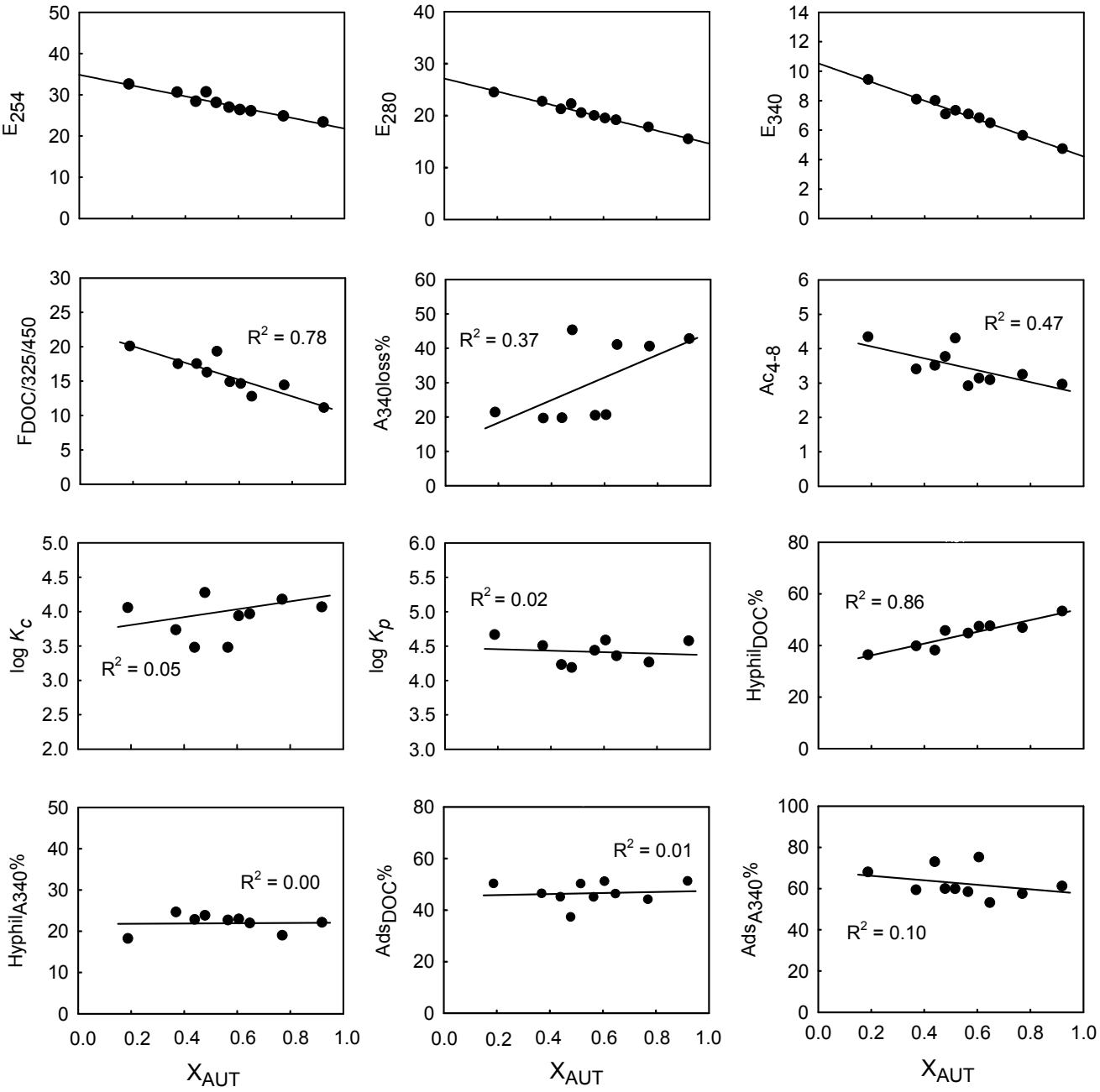

E254
E280
E340
FDOC/325/450
A340loss%
Ac4-8
log KC
log KP
HyphilDOC%
HyphilA340%
AdsDOC%
AdsA340%
J F M A M J J A S O

515

Fig. 2

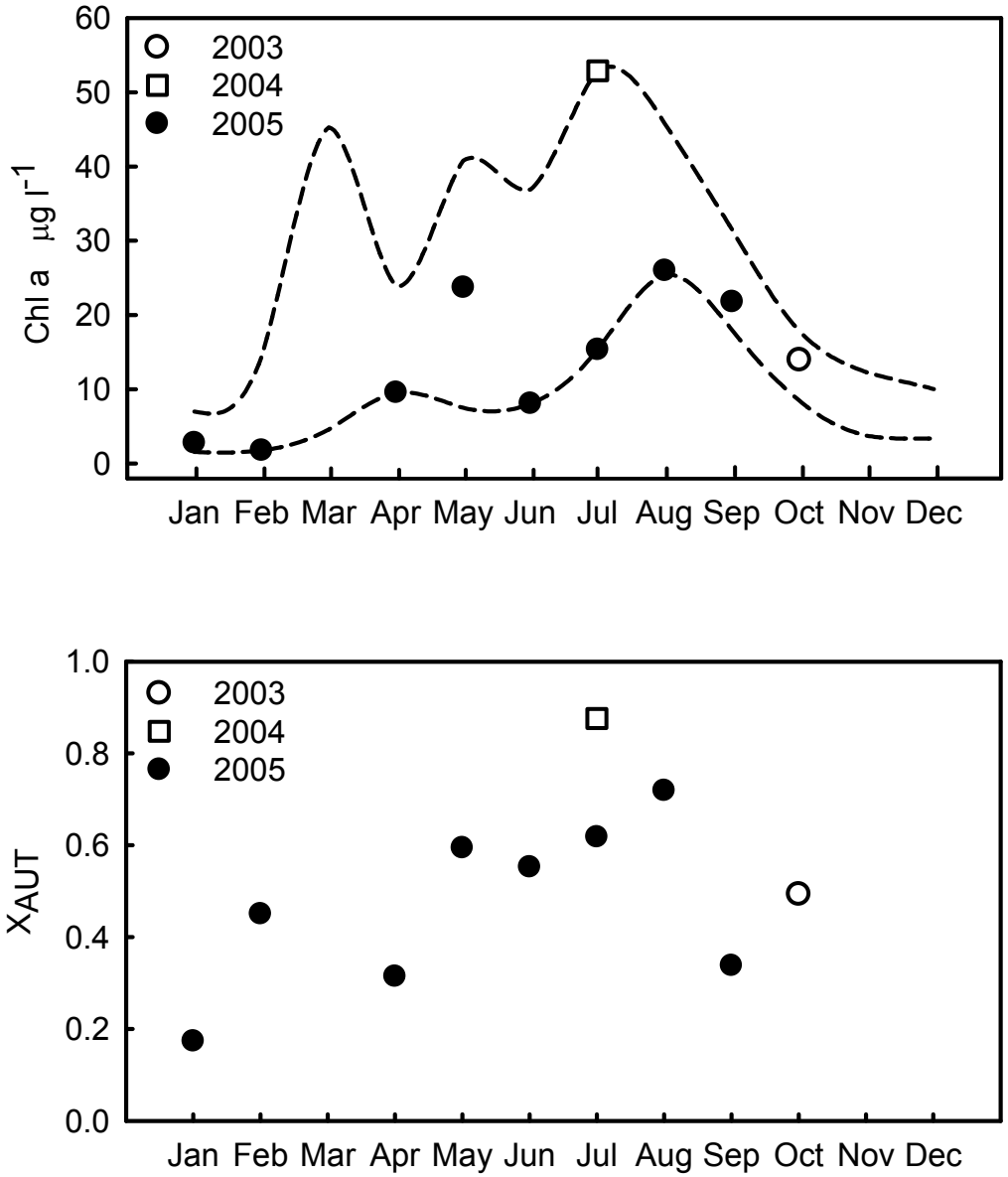
516

517



518

Fig. 3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40
30
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10
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Chl a µg l-1
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2004
2005
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec
1.0
0.8
0.6
0.4
0.2
0.0
XAUT
2003
2004
2005
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec

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520

Fig. 4