

Dear Editor,

We thank you and the reviewers for your careful reading of our manuscript entitled “Spatio-seasonal variability of chromophoric dissolved organic matter absorption and responses to photobleaching in a large shallow temperate lake”. Here, we submit the revised version of it for consideration to publication. The manuscript has been prepared in accordance with the Instructions for Authors and none of the authors have any conflicts of interest.

Following we will address all referee’s comments organized such that first the reviewer comments are given in regular font, directly followed by our response in red and in italics. Finally, you can find a list of the most relevant changes made in the manuscript, and a marked-up version of it.

Reviewer comments appear as normal text

*Our responses appear in red and italicised*

General comments. This paper documents variability in DOM along a longitudinal transect in Lake Balaton during 2014. The authors report that DOM concentrations generally ranged from 8 to 16 mgC/L in surface waters (which is relatively high), with values above 10 mgC/L mostly in the eastern end of the lake which receives inflow from a large wetland. They also report four optical properties of the DOM (absorbance, spectral slope coefficient, SUVA and E2/E3) as potential indices of DOM source or internal processing (especially photodegradation). Unfortunately, the choice of wavelengths for some of the optical measurements was not optimal, thus limiting comparison with other systems and studies.

*We agree with the reviewer that this can limit the comparison with other systems and studies, however, and as justified after in the text, this paper is strongly motivated by the understanding of the changes on the inherent optical properties of CDOM particularly with reference to the implications for remote sensing. In this framework, remote sensing studies often use 412 nm or 440 nm (Carder et al. 1989; Nelson et al. 1998; Schwarz et al. 2002) to describe CDOM absorption because information in the UV cannot be obtained from space. For this reason, and to ensure consistency with previous publications, our results are more strongly based on absorption in the blue rather than the UV*

Although the paper reports apparently new information about Lake Balaton, there are several matters in the text, tables and figures that will require re-thinking and major revision.

*We wish to thank the reviewer for this very thorough and constructive review. We agree with most of the suggestions and have therefore modified the text according to them. We think they have greatly enhanced the quality of the paper*

Specific comments.

1. Abstract. Line 35. The data on Fig 9c and 9d are not convincing evidence that UV irradiation caused any change in the absorbance of “autochthonous” DOM. Omit sentence

*The sentence has been omitted as suggested*

2. Abstract. Omit last two sentences. Nothing new here.

*The sentence has been omitted as suggested*

3. Lines 70-73. Unclear sentence. What nutrients are we talking about? Re-think and re-write.

*We agree it sounded like an incomplete sentence and have therefore rephrased it to now read as:*

*“CDOM can be remineralised by bacteria acting as a source of inorganic nutrients (Buchan et al. 2014), which is important for phytoplankton nutrition”*

4. Line 93. The wavelength 440nm is not commonly used for CDOM. 440nm is routinely used for Chl-a because it is the absorbance maximum for that pigment. The preferred wavelength for CDOM is in the UV, usually less than 370nm. Choice of 440 (blue) rather than UV needs to be justified.

*As justified later in the text (lines 196–198) and explained before, the fact of this study being strongly motivated by the understanding of the changes on the inherent optical properties of CDOM particularly with reference to the implications for remote sensing and to ensure consistency with previous publications, our results are based on CDOM absorption measurements at 440 nm*

5. Line 95. The spectral slope coefficient recommended by Helms et al (2008) and by Fichot and Benner (2012) is calculated over the UV range 275-295 nm. But in Balaton, the authors used a very different range (350-500 nm). Choice of this range needs to be justified given that the authors cite Helms et al and Fichot and Benner.

*We agree our choice needs to be justified and as modified in the text (line 201-203): “This range of calculation was consistent with Babin et al. 2003 and Matsuoka et al. 2012 amongst others and is more relevant to remote sensing studies than the use of wavelength ranges that extend into the UV spectrum”*

6. Line 113-120. Not true. There is a relatively rich literature on DOM in temperate lakes. Literature search needed.

*Modified in text as suggested (lines 114-115): “There is a relatively rich literature on DOM in temperate lakes (e.g., Zhang et al. 2011; Read & Rose 2013; Müller et al. 2014) but few studies have focused on large shallow lakes like Lake Balaton with a strongly continental climate and hence our understanding of the variability in CDOM optical properties in these systems is comparatively poorer*

7. Lines 124-127. For objective #1, add “over the course of one year.” Done For objective #3, since there were no direct measurements of the underwater light field (e.g.Kd PAR, or spectral Kd), this is not a bona fide objective.

*We agree the fact of not providing measurements of the underwater light field makes objective 3 unreachable and this has therefore been deleted*

8. Line 174-175. One year of data is not sufficient to document a representative seasonal cycle. Caveat needed.

*A clarification has been written specifying the seasonal variability was recorded and studied over the course of seven months (March to September 2014)*

9. Line 180. A 0.7µm filter will not remove small phytoplankton, bacteria or large organic colloids. Explain why 0.2µm filters were not used, and what the consequences of including small particulates might be.

*In response to the reviewer's suggestion, the use of 0.7 µm filters has been justified in the text (lines 178-183). They were selected for DOC measurements because of their compatibility with other measurements in POC (interesting in partitioning dissolved and particulate), chl- a, TSM, PC etc. Due to their larger nominal pore size, GF/F are expected to allow higher number of bacteria, viruses and colloids, which are not considered dissolved to pass through. This could lead to an overestimation of the DOC in the water samples. However, the differences due to filter are expected to be small and its importance is lessened given that DOC was only used to correlate with  $a_{CDOM}$ . Also due to their design GF/F normally retain particles smaller than is mentioned by the manufacture*

10. Lines 211-212. Helms et al (2008) recommended S275-295 as an indicator of in situ DOM photoprocessing for future DOM studies (not E2:E3). That needs to be acknowledged.

*We agree with the reviewer and this text has been deleted to avoid confusion*

11. Section 2.5. CDOM photodegradation. Except for Comment #13 (below), this is a very interesting and well planned experiment. Kudos.

*We very much appreciate the reviewer's comments on the merits of the CDOM bleaching experiment*

12. Lines 226-234. In nature, autochthonous DOM is more likely the exudates from live phytoplankton rather than dead cell remains (which would be colonized by microbes and sink out). Since exudates would be in the supernatant not the pellet, the method of grinding and digesting the pellet needs to be justified.

*We partially agree with the reviewer, cell exudates are an important source of autochthonous CDOM but other natural processes such as grazing by zooplankton, (Levine et al. 1999) or the presence of viruses causing the lysis of phytoplankton cells (Suttle et al. 1990) can also result*

*in release of CDOM. The process of pelleting, cleaning and breaking cells helped to mimic the effect of these processes and also ensure sufficient CDOM was produced for our experimental needs. The optical properties of the resulting CDOM were in line with expectations for autochthonous material*

*In the text we will comment on that fact the physical breaking cells has the potential to release cellular material that might not be excreted the optical properties of the material produced was consistent with that for autochthonous CDOM. Moreover, this material can also be released by cell lysis due to grazing or viruses in the natural environment (Levine et al. 1999, Suttle et al. 1990)*

13. Section 3.1. Seasonal variability. Caveat needed to acknowledge that seasonal changes were characterized for only 1 year, and may not represent the “average” seasonal cycle across multiple years with different weather patterns

*As suggested by the reviewer, it has been stressed in the text that seasonal changes were only measured for one year and therefore may not represent the typical seasonal cycle observed over longer time period*

14. Line 263. Rephrase sentence. With the exception of STO. Basin 1, the data on Fig 2 do not indicate high CDOM variability across most of the lake. In 5 of the 6 basins, DOC and  $a(440)$  were relatively stable.

*We agree with the reviewer that this was unclear, and the sentence has been clarified*

15. Line 286. The evidence for two peaks in DOC on Fig 2 is weak at best. Rephrase.

*We accept the reviewer’s comment here and have revised the text here accordingly. There is evidence of two peaks at the inflow (summer and autumn) with the latter coinciding with the timing of peaks elsewhere. However, the evidence is weak for more than one elsewhere*

16. Line 296. Add “during July when interbasin differences were likely to be highest.”

*Accepted and modified as suggested*

17. Lines 303 to 308. This paragraph needs to be re-thought. The range of  $S_{CDOM}$  across stations is actually quite wide in the scheme of things for natural waters. Compare to Helms et al (2008) and other studies where  $S_{CDOM}$  gradients have been reported.

*We appreciate the reviewer’s comment about the significance of these results and have modified the text to better emphasise the significance of the variability observed in  $S_{CDOM}$*

18. Line 318. Re-think. Concluding that DOC varied more than these two optical properties seems a consequence of scaling rather than a property of the variable. Comparison of CVs or Z-scores is needed here

*Reviewer 1 is correct and actually DOC showed greater variability through the system than for  $S_{CDOM}$  ( $CV = 0.053$ ) but smaller than for  $a_{CDOM}$  (440) ( $CV = 2.065$ ). Sentence has been correct accordingly to this*

19. Section 3.3. Photodegradation experiment. This section needs to be re-written. The results shown on Figure 9 indicate that UV irradiation had a significant effect only on the absorbance of allochthonous cdom. This result is consistent with the data on Fig 10a. That's all that one can say with confidence about the photodegradation experiment. There is no discernible effect on Scdom or on "autochthonous" cdom in these data.

*We agree with the reviewer and the sentence referring to autochthonous CDOM has been deleted*

20. Section 4. Discussion. This section has some overstatement, speculation and misinterpretation that needs to be removed.

*We agree with the reviewer with some over-reaching statements and misinterpreted results and have therefore removed several statements along the section and toned down others*

a. L 356-363. Actually, DOM across most of the lake is relatively constant (Fig 2&5c), as observed in other large lakes. High DOM in Balaton was restricted to stations near the inflow from a high DOM river. In studies of other large lakes, such stations were likely not sampled because they skew the data and contribute little to the total lake mass of DOM.

*We appreciate the reviewer's comments and agree that the variability observed in the western basin was not observed over the rest of the lake. However, the influence of the DOM entering the lake from the River Zala at times stretches across the western basin, which equates to an area of approximately 100 km<sup>2</sup>. The high concentrations of DOM observed in the west are therefore not an insignificant component of the total lake DOM. Moreover, the high input of DOM will certainly influence biological processes in the highly productive western part of the lake. We have amended the text here to emphasise that marked variability was confined to the western part of the lake*

b. L364-374. It is an exaggeration to say that  $a_{CDOM}$  and DOC varied seasonally throughout the system. This was only true for Basin I. In the other 4 basins,  $a_{CDOM}$  and DOC were relatively constant throughout the year (FIG 2)

*The text here has been toned down in line with the reviewer's comments*

c. L413-424. Photomineralization would not affect Scdom, it would only affect DOM concentration. But photobleaching can have a large effect on Scdom (e.g. Helms et al, 2008), and the data for Lake Balaton shown on figs 9 and 10 suggest that the humics are the fraction of DOM that is being bleached.

*This sentence has been revised such that it no longer implies that mineralisation influences SCDOM and rather emphasises that the effect is on the DOM pool more broadly*

d. L457. Again, microbes mineralize (respire) DOM or turn it into biomass rather than bleaching it

*We agree with the reviewer and the text has been modified as suggested*

e. L471-480. This is an over-interpretation of the results shown on Fig 9. It is unsupported speculation. Omit paragraph

*Paragraph has been omitted as suggested*

f. L481-491. This is also over-interpretation and speculation. In fig 9c, there was no difference between the irradiated “autochthonous” samples and the dark controls. In 9b, there were no controls. Omit paragraph

*Paragraph omitted as suggested*

g. L492-500. The low fluorescence signal from “autochthonous” DOM is likely due to its low concentration. Re-write

*We agree with reviewer's 1 suggestion and the sentence has been re-written*

h. L 505-519. Absent measurements of the underwater light field in the lake, it is speculation to propose light limitation by DOM in such a shallow, well-mixed and eutrophic lake. Ten percent of incident solar irradiance is generally sufficient to support strong phytoplankton growth, and the authors would need to show that attenuation by DOM was sufficient to reduce downwelling light penetration below that level in the epilimnion. Re-write paragraph

*High DOC in the western basin certainly impacts the quality and quantity of light available for photosynthesis but the authors acknowledge we cannot demonstrate that light limitation of primary production occurs near the inflow of the River Zala from the data presented in this paper. We have revised the text to reflect this*

21. Table 1. Change notation to (mean±SD). Specify date range in heading

*Table 1 has been modified as suggested*

22. Table 2. Are these data for July 2013 or July 2014 (or both)? If both, how was interannual variation accounted for. Specify dates in heading

*Specifications have been made on Table 2 as suggested*

23. Fig 2. Check the trace for  $a_{CDOM}$  (440). It behaves weirdly during autumn in some of the plots. Needs to be fixed

*Graphs have been corrected as suggested*

24. Fig 5. Add “measurements made in July...” to legend.

*Changes have been made to legend of figure 5 as suggested by Reviewer 1*

25. In Fig 5c the 5 DOC categories exaggerate actual spatial variability. The three highest categories are separated by less than 1 mgC/L, while the low category spans more than 8 mg.C/L. The category indicated by a yellow X is highly questionable (zero DOC? I don’t think so). Re-think this.

*Reviewer’s 1 comment made us realise some typographical error involved in the figure preparation and have been corrected*

26. Fig 9. Panel 9d is superfluous. Omit

*We agree with reviewer’s comment and the panel has been deleted*



**Anonymous Referee #2**

Reviewer comments appear as normal text

*Our responses appear in red and italicised*

C1

General comments: The study has investigated the spatial and seasonal variation of chromophoric DOM in a lake at several stations leading away from an inflow from a River supplying allochthonous DOM. Various methods were used to capture change in DOM over time and spatially including analysis of the absorption coefficient spectral slope, E2:E3 ratio, SUVA and Spectral Fluorescence signals. The study shows that the absorbance coefficient and DOC values were highest and most variable near the inflow to the lake, whereas other basins further located from the inflow had more stable values with more seasonal variability in the spectral slope of CDOM. While the study has performed what seems like a well-planned study there are some very strong statements that over reach the scope of the study. I suggest major revisions especially of the discussion.

*We very much appreciate anonymous referee #2 for the time and effort put into reviewing this manuscript, his / her comments have greatly contributed to improve the manuscript*

C2

Specific comments:

1. Line 84: What platforms are these? Please explain.

*Here we refer to the Sentinel-2 and Sentinel-3 satellites. This can easily be clarified*

2. Line 86: Please remove “the” in the part of the sentence which reads “However, CDOM is the arguably most challenging...”

*Modified as suggested*

3. Line 118: There are quite a few studies on temperate lakes, including seasonal work, see Müller et al 2014 “Hourly, daily and seasonal variability in the absorption spectra of chromophoric dissolved....”

*We agree with reviewer 2 and sentence has been modified as suggested and some references added. Sentence now can be read as: “There is a relatively rich literature on DOM in temperate lakes (e.g., Zhang et al. 2011; Read & Rose 2013; Müller et al. 2014) but few studies have focused on large shallow*

*lakes like Lake Balaton with a strongly continental climate and hence our understanding of the variability in CDOM optical properties in these systems is comparatively poorer. “*

4. Line 174: Mention the time of the year samples occurred to represent seasonal variability.

*This change has been addressed for reviewer’s 1 comment and can now be read as: “In order to capture seasonal variability in CDOM quantity and quality, water samples were collected fortnightly at 6 long-term monitoring stations on Lake Balaton over the course of seven months (March to September 2014) “*

5. Line 187: Instead of referring to the summer campaign as “intensive summer campaign” change to “spatial variability” in the whole manuscript.

*We agree with reviewer’s 2 suggestion and the change has been made effective on the whole manuscript*

6. Lines 190 and 193: Explain why two different instruments were used for the different campaigns.

*The samples were analysed at different institutions with different instruments for practical reasons. This has been clarified in the text*

7. Line 194-195: It is not clear when the reference sodium azide was used. Please make this clear.

*It was added immediately to preserve the samples; this has been clarified in the text*

8. Line 220: What was the temperature in the lake during this 7-day incubation?

*The mean temperature of the lake is now stated in the methods*

9. Line 211: Instead of writing “this wavelength” specify which wave length “this” refers to.

*We agree with reviewer 2, his sentence was confusing and has therefore been deleted*

10. Line 233: Was CDOM measured to know the start value? Give further explanation.

*This data has now been added as suggested*

11. Line 263: A seasonal variability in aCDOM, which was used to determine change in CDOM quantity, is not clear from figure 2. Either present statistical data backing this up or rephrase the statement.

*We have re-written the statement as suggested*

12. Line 263-269: The seasons are not shown in table 1, so the information that is referred to cannot be seen in the table. Add this information to the table.

*We agree with reviewer’s 2 observation and consider useful to include this information, therefore, the table has been modified to indicate the month of sampling*

13.Line 270: What is the relevance of comparing August values of sCDOM with June? Do the authors mean that these are the lowest and highest values? Please make this statement clearer.

*Yes, this is correct and is now acknowledged in the text*

14.Line292: When stating something is significantly lower the statistical data must be presented. Please add this data.

*Values of significance have been added*

15.Line295: This sentence regarding figure 4 does not present data and should be part of the methods section.

*This has been moved to the methods*

16.Line301: Are the values of min and max mentioned in the text also in the table? Please review.

*They are mentioned twice and indeed replicated, the text was therefore redundant and has been deleted*

17.Line304: can this statement that there was a marked variability be made with a change of what seems to be of 0,002 on a nm scale?

*We agree with reviewer's 2 comment and have therefore modified the sentence in the text so now it can be read: "In Keszthely (I) basin and the western parts of Szigliget (II) basin nearest the inflow of the Zala River, higher variability was observed with lower SCDOM coefficients more than elsewhere in the lake"*

18.Line 309: Where is the statistical data backing up the statement that it "varied significantly"? Please add this data.

*The text has been modified to avoid any claim of statistical significance but ranges are reported for easy comparison to previous studies*

19.Line 310-311: Refer to table 2 for the SUVA data.

*Modified as suggested*

20.Line 314: this statement about DOC data availability should be in methods since this cannot be seen in table 2 it is misleading to refer to it.

*We agree with reviewer 2 and the statement has been moved to the methods section*

21.Line 319: Is the correlation significant for all basins? It seems like Basin I has a strong correlation, how would it look like if they were analysed separately?

*The relationship was only significant for the Keszthely basin. In the other basins, the variability in DOC was much lower and the sample size was small. This is now clarified in the text*

22.Line 324: where is the data for these “marked alterations”? Does this refer to aCDOM? Rewrite and connect the sentences better.

*We agree this paragraph was confusing, it has been modified and a reference to figure 9 has been added*

23.Line 325: What was the temperature during this incubation? Can you really be sure that there was no bacterial degradation, 7 days is a long time for bacteria to degrade

*The mean daytime lake temperature is now specified in the manuscript. We do not state there was no bacterial degradation in the treatments only that the control samples suggest bacterial degradation was minimal over the experiment and certainly a minor influence compared to UV bleaching. It possible that bacterial degradation could have been enhanced in the light but it is improbable that this would explain the differences observed between the controls and treatments. The manuscript has been revised accordingly*

C4

DOM although you filtered through 0.2µm there are always some bacteria that are small enough to get through and grow to higher abundance over time, perhaps a portion of the DOM that cannot be measured with aCDOM was taken up like what is shown in figure 10?

*0.2-micron filtration typically removes >99% of bacteria from samples (Logan et al. 1993). However, it is unlikely that the filtered CDOM samples were axenic and as such it is possible that bacteria growth and metabolism of DOM in the samples contributed to the degradation of CDOM. However, comparison between the control and experimental samples clearly shows that the degradation of CDOM was greatly enhanced under solar radiation due to photobleaching*

24.Line327: where the reductions statistically significant?

*Statistical data have now been included (ANOVA,  $p < 0.01$ )*

25.Line330: Why was there an increase in the dark controls? Please discuss this.

*As now stated in lines 493-495: “The initial decrease in slope during the early part of the experiment echoes observations by Yamashita et al. (2013) and Fichot & Benner (2012) who*

*attributed this phenomena to microbial degradation of bioavailable CDOM (Nelson et al. 2004)”*

26.Line337: When stating no “significant variation” this implies statistical significance and thus data has to be presented. Present statistical data.

*This text has been re-written to avoid confusion*

27.Line341: same requirement as the previous comment. Show statistical data.

*Data added as requested*

Discussion section:

28.Line 362: Why is it surprising that the range has not been captured in the northern latitudes? Please explain.

*Northern boreal lakes generally have high CDOM concentrations (Curtis, 1998) and one would expect the range in these lakes to exceed that observed in Lake Balaton where catchment soils are less organic than in the peat dominated catchments of the boreal zone*

29.Lines 364-365: This statement is contradictory to your results. From figure 2 it rather seems like the aCDOM and DOC values were quite stable in most basins with variation only in Basin I at station 1 probably due to the inflow of the Zala River. Please re-write this part.

*We agree with reviewer 2, this paragraph was confusing, it has been re-written being now: “The seasonal pattern in CDOM absorption and DOC concentration varied considerably in the western basin, but was relatively constant in other basins. The annual peak(s) in aCDOM (440) and DOC occurred in spring and/or autumn some stations (e.g., ST03, ST12, ST30) were broadly coincident with or lagged slightly behind the annual runoff maxima suggesting a seasonal trend that was partly driven by the flushing of organic matter from catchment soils during high flow events. This pattern is common in many temperate and boreal lakes where DOC export from catchments is driven by the availability of flushable terrestrial carbon sources and the seasonality of precipitation and/or snowmelt”*

30.Lines 365-367: This correlation was not shown in the results and also does not seem consistent in all basins. Where is the data for this statement?

*This text has been re-written to avoid confusion*

31.Lines 369-373: Re-write this statement since it bases its argument on the previous statement that there could be coupling between aCDOM and DOC due to rainfall events, which was not observed in this study.

*The peaks in aCDOM and DOC at some stations occurred in spring or autumn when runoff was high. The clear exception to this trend was the stations located near the inflow where peak aCDOM occurred in the summer due to inputs from the Kis Balaton wetland. The text has been revised to emphasise that not all stations exhibited a seasonal trend that was driven by rainfall and runoff*

32.Line 372: Isn't the Keszthely basin the same basin that is closest to the inflow of the Zala River and thus repeating what was stated in the previous sentence?

*This has now been deleted*

33.Line 385-386: Please add the reference for the water residence time.

*Reference has been added as suggested*

C5

34.Line 395: Please add the statistical data to back-up the statement made that it was "significantly higher".

*Statistical data has been added as requested*

35.Lines 395-397: This information belongs in results since it is not a discussion.

*We agree with reviewer 2 and the sentence has been moved to results as suggested*

36.Line 398: Which studies are referred to in the statement "these studies"?

*We agree with reviewer 2 the sentence was lacking information, therefore. It was and the references of the studies added*

37.Line 418: Some references needed here about photobleaching and sCDOM, this sentence seems lost here.

*References have been added to support the statement*

38.Line 428-429: Please complete the sentence "influenced by both the provenance and subsequent transformations...." of what?

*Further detail is now provided in the text*

39.Lines 454-457: I'm not convinced that this was due to photobleaching, this section refers to figure 2, however this figure does not back-up this claim how do you rule out a dilution effect? Re-phrase.

*We agree with reviewer 2 this sentence was misleading; the statement has been re-written*

40.Line 461: Please add a reference to this paragraph.

*References have been added to support the statement*

41.Line 465: This data needs to be compared with the control and statistical confirmation presented in the results section.

*Comparison has been presented in the text as suggested*

42.Line 481: Here if referring to allochthonous it should be less susceptible instead of more. Please change.

*Paragraph modified*

43.Line 481: There is no visible change in SCDOM in the ALLO-CDOM. Please rephrase this statement

*We agree with the fact that there was not visible change in SCDOM for the allochthonous samples, the statement re-phrased stressing the fact that both the spectral slope and absorption coefficient for autochthonous CDOM were lower than for allochthonous samples*

44.Line 481-482: Where is the statistical data to back-up the claim of statistical significance? Is this a comparison between allochthonous with autochthonous or with start values? Please add the data to the results section and re-phrase this discussion based on this.

*Data added as requested*

45.Line 492: Where is the data for fluorescence spectra of autochthonous material? Figure 10a and 10b only present allochthonous

*There were more than ten orders of magnitude difference in fluorescence intensity between CDOM allo and CDOM auto samples, presumably driven by the difference in concentration. Given the low concentrations of CDOM, after Milli-Q correction, there was no measurable fluorescence signal for the autochthonous samples. Therefore, fluorescence spectra of autochthonous material have not been presented in figure 10*

C5

46.Line 495: where is this data?

*Please refer to response above*

47.Line 500: could this loss not be due to bacterial degradation?

*Indeed, we suggest that this loss is due to photobleaching and not to bacterial degradation. Statement stressed in the text to avoid confusion*

48.Line 505-506: Please add a reference to this sentence.

*References have been added to support the statement*

49.Line 506: what is meant by “elsewhere”?

*Paragraph has been re-written*

50.Line 505-509: This is a very strong statement that cannot be proven with the data from this study.  
Please re-write.

*We agree and have toned down the statement in line with the reviewer’s comments*

51.Line 512: Also this statement is too bold since this was not within the scope of this study.

*Statement deleted*

52.Line 522-524: Please add a reference to this statement.

*Statement deleted*

53.Line 547: Isn’t the contribution of wetlands well known? Remove “novel”.

*Removed as suggested*

Technical comments:

54.Line 70-71: Please review this sentence, it seems like information is being repeated and there is a misuse of the word “whilst”.

*Sentence has been re-written as suggested by reviewer’s 2*

55.Line 71: In the same sentence as the above comment “...this fulfilling important role...” should probably be “thus fulfilling an important role”.

*Sentence re-written as suggested by reviewer’s 2*

56.Line 75: can CDOM have a behaviour? Perhaps property could be used instead.

*Sentence re-written as suggested by reviewer’s 2*

57.Line 87: should be changed to “for reliable estimation of remotely...” Please change.

*Sentence re-written as suggested by reviewer’s 2*

58.Line 89: should be changed to “studies have explored the application...”

*Sentence re-written as suggested by reviewer’s 2*

59.Line 97: change to “size of DOM molecules...”

*Sentence re-written as suggested by reviewer’s 2*



60.Line 98: I think the authors mean larger/greater molecules, not higher.

*This text has been re-written to avoid confusion.*

61.Lines 131-133: Please add references to this information about the study area.

*References have been added as suggested*

62.Line 136: should be changed to "...at that time of the year..."

*Sentence has been changed as suggested*

63.Lines 162-164: Please add a reference to this statement.

*References have been added as suggested*

64.Line 165: what is meant by "...less noticeable..."? Less than what?

*This text has been re-written to avoid confusion*

65.Line 219: I suggest moving "fifty-six" to Line 222 so it reads "Fifty-six samples were taken in total of which 21 were composed of..."

*Sentence changed as suggested*

66.Line 228: Please add a reference to the dominance of the phytoplankton in this particular lake.

*References have been added as suggested*

67.Line311: if reference to figure 6d and 6e is made then SUVA should be mentioned first and then E2/E3 ratio to be consistent. Then you can say that it refers to those figures respectively.

*Sentence modified as suggested*

68.Line315: mean value in table 2 for Keszthely basin is 9.66 not 9.67 as it says in the text. Which is correct? Please review.

*Sentence modified to 9.66, corrected values*

69.Line317: Do you mean with increasing distance from Zala River?

*Sentence modified to avoid confusion*

*Yes, DOC concentrations slowly decreased with increasing distance from Zala River*

70.Line 317: remove "in" before the word similarly.

*Sentence changed as suggested*

71.Line346: Change to "there were more than ten orders..."

*Sentence changed as suggested*

**Reference list:** I have not checked the reference list.

72.Line434: How does this statement connect with the data in this study: “previous studies have also found marked differences in the E2:E3 between natural waters...” Present the data from the study and then connect with what other studies have found.

*Data from the study is shown in results, lines 320-321 and Table 3. Statement modified to avoid confusion. References added to support the statement*

73.Line446: Remove “in” after Lake Balaton.

*Sentence changed as suggested*

74.Line450: change “sensitive” to sensitivity.

*Changed as suggested*

75.Line456: Add: and, between the two ranges.

*Changed as suggested*

76.Line530: change to “new approaches are needed...”

*Sentence modified as suggested*

77.Tables 1 and 2: Is there a reason why values are stated as Max-Min instead of Min-Max? Consider changing to better fit with standard way of reporting such values.

*Modified as suggested*

78.Figure2: the lines connecting data points for aCDOM seem to connect in a strange way or to be disconnected. Please review and fix. Figure 9: add to legend what “DC” refers to, dark control?

*Figure modified as suggested*

**List of relevant changes in the manuscript**

1. Improvements in grammar and writing as mentioned by the referees
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# Spatio-seasonal variability of chromophoric dissolved organic matter absorption and responses to photobleaching in a large shallow temperate lake

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## Abstract

The development and validation of remote sensing-based approaches for the retrieval of CDOM concentrations requires a comprehensive understanding of the sources and magnitude of variability in the optical properties of dissolved material within lakes. In this study, spatial and seasonal variability in concentration and composition of CDOM and the origin of its variation was studied in Lake Balaton (Hungary), a large temperate shallow lake in central Europe. In addition, we investigated the effect of photobleaching on the optical properties of CDOM through in-lake incubation experiments. There was marked variability throughout the year in CDOM absorption in Lake Balaton ( $a_{\text{CDOM}}(440) = 0.06 - 9.01 \text{ m}^{-1}$ ). The highest values were consistently observed at the mouth of the main inflow (River Zala), which drains humic-rich material from the adjoining Kis-Balaton wetland, but CDOM absorption decreased rapidly towards the east where it was consistently lower and less variable than in the westernmost lake basins. The spectral slope parameter for the interval of 350–500 nm ( $S_{\text{CDOM}}(350-500)$ ) was more variable with increasing distance from the inflow (observed range 0.0161-0.0181  $\text{nm}^{-1}$  for the mouth of the main inflow and 0.0158-0.0300  $\text{nm}^{-1}$  for waters closer to the outflow). However, spatial variation in  $S_{\text{CDOM}}$  was more constant exhibiting a negative correlation with  $a_{\text{CDOM}}(440)$ . DOC was strongly positively correlated with  $a_{\text{CDOM}}(440)$  and followed a similar seasonal trend but it demonstrated more variability than either  $a_{\text{CDOM}}$  or  $S_{\text{CDOM}}$  with distance through the system. Photobleaching resulting from 7 days exposure to natural solar UV radiation resulted in a marked decrease in allochthonous CDOM absorption (7.04 to 3.36  $\text{m}^{-1}$ , 42% decrease). Photodegradation also resulted in an increase in the spectral slope coefficient of dissolved material.

## **1. Introduction**

### **1.1. Importance of CDOM in lakes**

There are approximately 117 million lakes on Earth greater than 0.002 km<sup>2</sup> in surface area collectively covering about 3.7 % of its non-glacial surface (Verpoorter et al. 2014). The importance of the role that lakes play as regulators of the carbon cycle and thereby global climate has only recently been recognized (Tranvik et al. 2009), acting as both a sink (sediment storage through flocculation from dissolved to particulate organic carbon) or source for carbon (degradation and resulting mineralization to CH<sub>4</sub>, CO and CO<sub>2</sub>; Cole et al. 2007; Benoy et al. 2007; Tranvik et al. 2009). As a result they also play an important role in transforming and releasing terrestrially-derived carbon to the atmosphere and ocean (Tranvik et al. 2009). As extremely sensitive ecosystems (Millennium Ecosystem Assessment 2005; IPCC 2007), lakes can respond rapidly to external pressures including meteorology, climate and land use change. This has led to the emerging concept of lakes as sentinels of environmental change (Adrian et al. 2009; Williamson et al. 2009a; Schindler 2009; Williamson et al. 2009b).

The optical properties of lakes provide particularly useful metrics for measuring ecosystem change (Vincent et al. 1998) as they not only convey information on the quantity of particulate and dissolved material but also its quality (Williamson et al. 2014). Furthermore, understanding how the optical properties of particulate and dissolved material in lakes influences the underwater light field and water-leaving radiative signal is important for the development and application of remote sensing techniques for lake monitoring and assessment, but also their application to lake carbon studies.

Much of the dissolved organic matter (DOM) found in lakes typically represents between 90 to 100 % of the total carbon pool (Wilkinson et al. 2013) and is derived from terrestrial inputs, transported through streams, rivers and wetlands. This allochthonous component of the DOM originates from soils, sediments and plants and is primarily composed of humic substances. The autochthonous fraction of DOM is produced mostly by phytoplankton, zooplankton and bacterioplankton and is largely composed of fulvic acids, carbohydrates, amino acids, proteins, lipids and organic acids.

Chromophoric dissolved organic matter (CDOM) is the coloured fraction of DOM. It is one of the dominant colour-forming constituents in lakes: it not only exerts a strong influence over the underwater light field and water-leaving radiance but it also has a number of important ecosystem functions. First of all, it absorbs light strongly in the ultraviolet (UV) spectrum limiting the penetration of biologically-damaging UV-B radiation providing protection for phytoplankton and other primary producers (Hoge et al. 1995; Laurion et al. 2000; Zhang et al.

2007; Williamson et al. 2001). In addition, CDOM can be remineralised by bacteria acting as a source of inorganic nutrients (Buchan et al. 2014), which is important for phytoplankton nutrition, thus fulfilling an important role in the development of phytoplankton blooms and lake metabolism more widely. On the other hand, studies have also shown that light absorption by CDOM can reduce the amount and quality of photosynthetically active radiation (PAR) available to phytoplankton, thereby decreasing primary production and constraining lake metabolism (Kirk 1994; Laurion et al. 1997, 2000; Vähätalo et al. 2005). Moreover, its conservative properties with dissolved organic carbon (DOC), means CDOM is often used as a proxy for DOC. Thus, there is substantial interest in the use of CDOM as an optical tracer of DOC due to the importance of the latter in regulating physical, chemical and biological properties of lakes. It is therefore important that we develop a better understanding of the optical properties of CDOM and how these relate to the chemical composition and concentration of DOM whether driven by changes to source relationships or through the in-lake processes and transformation of the carbon pool.

Understanding how the optical properties of CDOM vary both temporally and spatially within lakes and how the observed variability influences the underwater light field is of particular importance for the development and validation of remote sensing-based approaches for retrieving CDOM concentrations. The recent launch of new satellite missions (e.g., Sentinel-2 and -3), allied with the prospect of new hyperspectral sensors (e.g., EnMAP), has provided a new impetus for the development and application of remote sensing techniques for the assessment and monitoring of inland water quality. However, CDOM is arguably the most challenging water quality parameter for reliable estimation of remotely sensed observations (Palmer et al. 2015) and, in spite of its importance to the physical, chemical and biological function of lakes, it remains one of the least studied parameters. Indeed, few studies have explored the application of remote sensing for the estimation of CDOM in lakes. To progress such research, an improved understanding of the spatial and temporal variation in the optical properties of CDOM in lakes is needed.

## 1.2. Optical properties of CDOM

CDOM concentration is commonly measured by its absorption coefficient ( $a_{\text{CDOM}}$ ) at 440 nm, whereas its structure and composition has been most commonly inferred from the spectral slope parameter ( $S_{\text{CDOM}}$ ) calculated between two reference wavelengths (Helms et al. 2008; Fichot & Benner 2012). Other optical metrics related to CDOM compositions include the E2:E3 ratio or M value, which is the ratio of absorption at 250 nm and 365 nm. De Haan and De Boer (1987) used E2:E3 ratio to track changes in relative size of CDOM molecules: increases in molecular

size result in decreases in the E2:E3 ratio because of stronger light absorption by higher molecular weight compounds at longer wavelengths.

In addition, Weishaar et al. (2003) introduced the specific UV absorbance parameter ( $SUVA_{254}$ ) defined as the UV absorbance at 254 nm normalised by the dissolved organic carbon (DOC) concentration.  $SUVA_{254}$  has been shown to be strongly correlated with DOM aromaticity in a large number of aquatic environments, with higher  $SUVA_{254}$  values indicative of a higher abundance of aromatic compounds. Previous studies have used  $SUVA_{254}$  to explore variability in the composition of DOM in natural waters.

The compositional properties of CDOM vary over time in response to processes such as microbial decomposition and exposure to UV irradiation. Previous studies have shown the latter process, first described by Wipple (1914) as ‘photobleaching’, plays a major role in the transformation of DOM in natural waters. Exposure to solar irradiance has been shown to reduce its capacity to absorb light, the loss of absorptivity is linked to a reduction in molecular weight (MW), alteration of its chemical composition and an increase in the bioavailability of DOM (Geller 1986; Keiber et al. 1990; Wetzel et al. 1995; Lindell et al. 1995; Corin et al. 1996; Reche et al. 1998) with implications for lake metabolism.  $S_{CDOM}$  is also known to vary in response to photobleaching (Swan et al. 2012; Fichot & Benner 2012).

Most previous studies on the origin, distribution and degradation of DOM and how this influences the optical properties of CDOM have been undertaken in oceans (Andrew et al. 2013; Matsuoka et al. 2014; Hancke et al. 2014; D’Sa et al. 2014), coastal waters (Stedmon et al. 2000; Vantrepotte et al. 2007; Kutser et al. 2009; Para et al. 2013) or in high latitude lakes (Ficek et al. 2011; Ylöstalo et al. 2014). Understandably, the bias towards high latitude systems partly reflects the fact this region contains a high density of humic-rich lakes. There is a relatively rich literature on DOM in temperate lakes (e.g., Zhang et al. 2011; Read & Rose 2013; Müller et al. 2014) but few studies have focused on large shallow lakes like Lake Balaton with a continental climate and hence our understanding of the variability in CDOM optical properties in these systems is comparatively poorer. In systems such as Lake Balaton, although the DOC pool is typically smaller than at higher latitudes it still plays a significant role in regulating light availability and therefore lake metabolism, while the influence of processes such as photobleaching is also likely to be more pronounced.

In this study we explore spatial and seasonal variability in optical properties of CDOM in Lake Balaton, a large temperate lake with a highly continental climate. We investigate how changes in spectral absorption, spectral slope coefficients,  $SUVA_{254}$  and the E2:E3  $raF_{tio}$  can be used to infer information on the concentration, source



and decomposition of CDOM. The main objectives of the study were to: (1) characterize the spatial and seasonal trends in CDOM in Lake Balaton over the course of a year and to (2) determine the origin and magnitude of the variability of different sources of CDOM.

## 2. Material and methods

### 2.1. Study site

With a surface area of 596 km<sup>2</sup> and a mean depth of 3.25 m, Lake Balaton in Hungary is the largest shallow lake in central Europe (Fig. 1a). The region is situated on the boundaries between the Mediterranean, continental, and oceanic climatic zones (Peel et al. 2006), resulting in a climate characterised by dry summers and moderately wet winters with typical continental extremes in temperature. The first two weeks of January are the coldest periods of the year (−4 – 3 °C) whilst July and August the warmest months (15 – 28 °C). The annual precipitation in the Lake Balaton region is between 500–750 mm; most precipitation falls during the spring, while the minimum occurs during the summer. There is a secondary maximum in autumn, due to a strong cyclone activity at that time of the year. In regards to solar radiation, Lake Balaton is situated between the southern, western and central Transdanubian regions in Hungary with an annual mean of 4500 MJ m<sup>−2</sup>. The highest solar radiation is received in July (650 MJ m<sup>−2</sup>), while cloudy weather and shorter days mean that lowest radiation occurs in December. The maximum in sunshine duration is also reached in July with more than 250 hours, falling to a minimum of approximately 40 hours during winter months.

Lake Balaton is usually divided into four basins (south-west to north-east): Keszthely; Szigliget; Szemes; and Siófok (Fig. 1b). The lake has 20 permanent and 31 temporary inflows, many of which are small streams or springs in the lake bed. The largest inflow to the lake is the Zala River, which flows through Kis-Balaton reservoir – a large semi-natural wetland system – and enters the lake in the westernmost part of Keszthely basin (Fig. 1b). The only outflow is the Sió channel in the northeast that connects the Siófok basin with the Danube River.

Lake Balaton has experienced eutrophication since the middle of the 18<sup>th</sup> century due to agricultural intensification and urbanisation within the catchment. Since the early 1980s, significant effort has been invested in improving its water quality (Tátrai et al. 2000). The construction of Kis-Balaton reservoir and wetland system was one of the main engineering controls built to reduce nutrient inflow from the Zala River and the overall loading within the lake. Kis-Balaton removes approximately 60 % of the annual nutrient loading to Lake Balaton (Szilágyi et al. 1990). However, nutrient inputs from the Zala River still result in high summer primary production in the eutrophic (>20 mg m<sup>−3</sup> chl-a) waters in the western basins, with a steep gradient towards more mesotrophic (2 -

20 mg m<sup>-3</sup> chl-a) waters in the east. The hypertrophic Kis-Balaton wetland system is believed to be responsible for much of the DOM entering the lake, largely derived from luxuriant growth and decomposition of aquatic plants. Previous research (Palmer et al. 2013; Riddick et al. 2015) has shown that CDOM is usually significantly higher close to Zala River inflow, and decreases towards the outflow but very little is known about the seasonal dynamics of CDOM in the system.

Suspended particulate matter in Lake Balaton is highly variable (spatially and temporally) due to its very shallow depth, constant mixing and susceptibility to wind-driven resuspension events (Istvánovics et al. 2004). Phytoplankton composition in the lake shows strong seasonal trends, with two annual blooms (Padisak & Reynolds 1998; Présing et al. 2008; Hajnal & Padisák 2008). In late summer and early autumn, cyanobacterial blooms often occur in the Keszthely basin (I), extending westwards to the Szigliget (II) and Szemes (III) basins and very occasionally to the Siófok (IV) basin (Padisak & Reynolds 1998; Présing et al. 2008; Hajnal & Padisák 2008). The lowest phytoplankton biomass generally occurs in February when the lake can be ice-covered; a small dinoflagellate bloom may also occur in April (Mózes et al. 2006).

## **2.2. Water sampling**

Spatial variability in CDOM quantity and quality was assessed over a 1-week period in July 2013 (6 stations) and a 3-week period in July 2014 (25 stations) at 31 stations over a biogeochemical gradient from the southwest in the water masses influenced by Zala River to the northeast near the outflow (Fig. 1c). Five stations were also sampled in the Kis-Balaton reservoir during the same period (2 in 2013 and 3 2014). These intensive sampling campaigns were timed to coincide with the annual summer peak in DOC to capture the maximum spatial variability likely to occur in the system.

In order to capture seasonal variability in CDOM quantity and quality, water samples were collected fortnightly at 6 long-term monitoring stations on Lake Balaton over the course of seven months (March to September 2014). These comprised stations 01 and 03 from Keszthely basin (I), station 12 from Szigliget basin (II), station 20 from Szemes basin (III) and stations 25 and 30 from Siófok basin (IV) (for location of stations see Fig. 1c).

Water samples for DOC analysis were collected in triplicate using acid-rinsed polypropylene bottles at 0.3 m depth below the surface. The samples were immediately stored on ice and in the dark until they were transferred to the laboratory for filtration. The samples were filtered through 0.7 µm pre-combusted 47 mm glass-fibre membranes (Whatman GF/F) and stored cold (4°C) and in the dark until measurement. Filters were selected for DOC measurements because of their compatibility with other POC measurements and were combusted reducing the possibility of contamination. Even though due to their large nominal pore size, these filters are expected to

allow high number of bacteria, viruses and colloids through, the differences with a smaller nominal pore size are expected to be small given that this DOC was only used to correlate with  $a_{CDOM}$ . Samples for CDOM analysis were collected separately in acid-rinsed amber glass bottles from 0.3 m depth and immediately stored on ice and in the dark until transfer to the laboratory. Samples were pre-filtered through pre-combusted 0.7  $\mu\text{m}$  pore size glass-fibre membranes (Whatman GF/F) to remove large particles and then re-filtered through a 0.2  $\mu\text{m}$  Whatman nucleopore membrane filters. The samples collected as part of the seasonal sampling campaign were measured fresh (i.e., without preservation) within 24 hours following Tilstone et al. (2002). The samples collected during the campaigns focused on spatial variability were preserved with a 0.5 % (vol:vol) solution of 10 g L<sup>-1</sup> of sodium azide (NaN<sub>3</sub>) (Ferrari et al. 1996) prior to analysis, which was completed within 1 month of sample collection.

### 2.3. CDOM absorption

The spectral absorbance ( $A$ ) of the seasonal samples was measured on a Shimadzu UV 1601 spectrophotometer (Cuthbert & Del Giorgio 1992) using a 1, 4 or 10 cm cuvette between 350 and 800 nm with a 0.5 nm sampling interval using ultrapure water (Milli-Q) as a reference (Vodacek et al. 1997). These samples were measured fresh in the laboratory of the Balaton Limnological Institute. Samples from campaigns focused on spatial variability were preserved immediately with 0.5 % (vol:vol) sodium azide and transported to the University of Stirling for analysis on a Cary-100 UV-visible spectrophotometer using a 1 or 10 cm cuvette between 200 and 800 nm with 0.2 nm sampling interval against ultrapure water with 0.5 % (vol:vol) sodium azide as the reference. The absorbance data were baseline corrected by subtracting the mean of  $a_{CDOM}$  in a 5 nm interval centred at 685 nm (after Babin et al. 2003). This wavelength was selected because absorption by CDOM and other dissolved constituents is negligible in the far red (Pegau et al. 1997). The CDOM spectral absorption coefficient ( $a_{CDOM}$ ) was calculated as follows (Kirk 2010):

$$a_{CDOM}(\lambda, \text{m}^{-1}) = 2.303 \cdot \frac{A_{CDOM}(\lambda)}{L} \quad (1)$$

where  $a_{CDOM}(\lambda)$  is the absorbance over a pathlength of  $L$  meters.

The absorption coefficient at 440 nm was used to express variation in CDOM quantity. This wavelength was preferred over UV wavelengths because it is more relevant to (and consistent with previous) studies on the optical properties and remote sensing of CDOM in natural waters (e.g. Carder et al. 1989; Nelson et al. 1998; Schwarz et al. 2002). The spectral slope for the interval of 350–500 nm ( $S_{CDOM}(350-500)$ ) (Babin et al. 2003) was determined by fitting a single decreasing exponential function to the absorption spectra using non-linear regression (Bricaud et al. 1981; Twardowski et al. 2004) between 350 and 500 nm, as follows:

$$a_{\lambda}(\text{nm}) = a_{\lambda_{\text{ref}}} \cdot e^{-S(\lambda - \lambda_{\text{ref}})} \quad (2)$$

where  $\lambda_{\text{ref}}$  is a reference wavelength (440 nm in this study). This range of calculation was consistent with Babin et al. (2003) and Matsuoka et al. (2012) amongst others and is more relevant to remote sensing studies than the use of wavelength ranges that extend into the UV spectrum.

The E2/E3 index was calculated as the ratio of the CDOM absorption coefficients at 250 and 365 nm. Previous studies have shown that decreases in this ratio are related to increases in molecular size, aromaticity and humification of DOC (Peuravuori & Pihlaja 1997). Finally, specific UV absorptivity at 254 nm (SUVA<sub>254</sub>) was obtained by normalising the absorption at 254 nm by the DOC concentration (mg L<sup>-1</sup>) (Weishaar et al. 2003).

## 2.4. Dissolved organic carbon (DOC)

Samples for dissolved organic carbon (DOC) were measured by thermal catalysis at 950°C in an Elementary High TOC instrument (Elementar Analysensysteme GmbH Germany) equipped with a platinum catalyst cartridge using synthetic air as the carrier gas. For spatial measurements, DOC data were only available for 18 out of 31 stations (Table 3).

## 2.5. CDOM photodegradation

In order to examine the effects of solar radiation on autochthonous and allochthonous CDOM in Lake Balaton, a 7-day in-lake incubation experiment was undertaken during mid-July 2014. CDOM samples from Lake Balaton were incubated in 65 mL capacity quartz tubes over 7 days under natural solar radiation. The mean daytime lake temperature of the lake over the experimental period was 24.6 °C. The quartz tubes were attached horizontally to a wire frame to minimise shading and submerged approximately 1 cm beneath the water surface in a sheltered bay. Fifty-six samples were taken in total of which twenty-one experimental were composed of phytoplankton-derived autochthonous CDOM (CDOM<sub>auto</sub>) and a further 21 were comprised of CDOM of allochthonous origin (CDOM<sub>allo</sub>). In addition, 14 dark control samples (CDOM<sub>allo-dark</sub> and CDOM<sub>auto-dark</sub>) were incubated (7 allochthonous, 7 autochthonous).

The autochthonous CDOM was extracted from a strain of *Cylindrospermopsis raciborskii* (ACT 9502) previously isolated from Lake Balaton and grown under nutrient replete conditions in semi-continuous culture at 24°C and 14:10 h light/dark cycle. *Cylindrospermopsis raciborskii* dominates the phytoplankton community during summer in Lake Balaton (Présing et al. 1996), often contributing >90% of the total biomass. A total of 3200 mL of cultured material was centrifuged in the early stationary growth phase (5 min, 4000 rpm; Hermle Z320 BHG) in order to

shake off the remaining allochthonous CDOM in the supernatant and the resulting cell pellet was broken using a mini-bead beater (30 s, 3500 rpm; Biospec products) to facilitate the release of cell contents. The total cell disruption was confirmed by microscopic examination (Olympus BX51). Similar processes take place in natural environments. During some natural process the cell releases its content, for example when grazed by zooplankton, (Levine et al. 1999) or in the presence of algal viruses causing the lysis of natural phytoplankton communities (Suttle et al. 1990). The material was incubated in the dark for 5 days at 20°C to allow production of CDOM and then diluted to 0.4 % (vol:vol) with ultrapure water. The  $a_{CDOM_{auto}}$  at day 0 was  $1.33\text{ m}^{-1}$ .

For the  $CDOM_{allo}$  samples, 5 L of water was collected inside the mouth of Zala River at 1 m below the surface with an acid-rinsed amber glass bottle on day zero of the experiment. The predominately allochthonous origin of the DOM was confirmed by mass-spectrometry (Lajtha & Michener 1994). 0.5 L of water was filtered in triplicate and the filter was dried and stored until analysis on an Isotope Ratio Mass Spectrometer (ANCA-MS, Europa Scientific Ltd., UK). The  $\delta^{13}C$  values of the allochthonous samples analysed had a mean  $\delta^{13}C$  signal of  $-33.48 \pm 0.43$ , which is consistent with published data on the  $\delta^{13}C$  signature of C3 plants.

The  $CDOM_{auto}$  and  $CDOM_{allo}$  samples were filtered through a pre-combusted 47 mm diameter glass fibre filter paper (Fisher Brand MF300, nominal pore size  $0.7\text{ }\mu\text{m}$ ) previously rinsed with ultrapure water to remove particulate matter including bacteria. The samples were re-filtered using  $0.2\text{ }\mu\text{m}$  porosity Whatman nucleopore membrane filters. The quartz tubes were acid-washed for 24 h, and then rinsed repeatedly with ultrapure water. The tubes were then filled with the CDOM samples and sealed with parafilm to prevent contamination. The dark samples were wrapped with black vinyl tape (resistant to UV radiation). Data on the total solar UV-radiation during the experiment were obtained from the Hungarian Meteorological Service.

One  $CDOM_{allo}$  and one  $CDOM_{auto}$  sample were collected and analysed in triplicate at daily time steps and their absorption and fluorescence spectra were measured. CDOM absorption coefficients were measured according to the methods detailed above. Subsamples for fluorescence measurements were stored cold ( $4\text{ }^{\circ}\text{C}$ ) and in the dark after preservation with a 0.5 % (vol:vol) of  $10\text{ g L}^{-1}$  sodium azide ( $\text{NaN}_3$ ) (Ferrari et al. 1996) until further analysis.

Spectral fluorescence signatures (SFS) were measured using an Instant Screener (ISC) analyser (Laser Diagnostic Instruments Ltd., Tallinn, Estonia). Measurements were made using a 1 cm quartz cuvette at excitation wavelengths from 240 to 360 nm and at emission wavelengths from 260 to 575 nm with a 5 nm slit-widths for excitation and emission wavelengths. Ultrapure water with 0.5 %  $\text{NaN}_3$  was used as a reference. The fluorescence signals of the samples were examined in two spectral regions. “Protein-like” fluorescence ( $F_n(280)$ ) was excited

at a wavelength of 280 nm, with the emission peak recorded in the range  $350 \pm 5$  nm. “Humic-like” fluorescence ( $F_n(355)$ ) was excited at 355 nm and its emission was measured at  $450 \pm 5$  nm (Vodacek et al. 1997; Vignudelli et al. 2004). The fluorescence data were expressed as QSU (Quinine sulfate units; Coble et al. 1998).

### 3. Results

#### 3.1. Seasonal variability

##### 3.1.1. CDOM optical properties

There was some (pronounced in basin 1, noticeable in basin 2 and low in basin 4) seasonal variability in the CDOM concentration measured in Lake Balaton throughout a year (Fig. 2, Table 1). It should be stressed that seasonal changes were only measured for one year and may not represent the typical seasonal cycle observed over longer time periods. High  $a_{CDOM}(440)$  values were observed throughout the year at the mouth of the Zala River in the Keszthely basin at ST01 (Table 2), with concentrations increasing from an annual minimum in spring ( $3.69 \text{ m}^{-1}$  in March) to a peak in August ( $9.01 \text{ m}^{-1}$ ) during the warmest and driest period of the year (Anda & Varga 2010). Values of  $a_{CDOM}(440)$  decreased for ST03 ( $0.64 \text{ m}^{-1}$  in June –  $1.43 \text{ m}^{-1}$  in March) and were consistently lower and less variable in the other lake basins with a maximum value of  $0.63 \text{ m}^{-1}$  observed at ST12 (Szigliget) in September and a minimum value of  $0.06 \text{ m}^{-1}$  at ST30 (Siófok) in June.

The lowest and highest  $S_{CDOM}(350-500)$  coefficients were observed in Keszthely (I) basin and ranged from  $0.0161 \text{ nm}^{-1}$  in August at ST01 to  $0.0221 \text{ nm}^{-1}$  in June at ST03 (Fig. 2).  $S_{CDOM}$  was more variable with increasing distance from the inflow; all the stations except for ST01 demonstrated a maximum in early or mid-summer month (June–July) and minima in spring and autumn (Table 2). The maximum value for  $S_{CDOM}(350-500)$  was  $0.0300 \text{ nm}^{-1}$  at ST30 in June and the minimum observed was  $0.0158 \text{ nm}^{-1}$  at ST30 in May, highlighting the high variability of this parameter near the outflow. The mean (and range) in  $S_{CDOM}(350-500)$  for Lake Balaton in the summer period was  $0.0211 \text{ nm}^{-1}$  ( $0.0174$  to  $0.0229 \text{ nm}^{-1}$ ), higher than values reported in several other spatial variation studies including Lake Erie (Binding et al. 2008;  $S_{CDOM}(350-400)$   $0.0161 \text{ nm}^{-1}$ ) and Lake Chapman (O’Donnell et al. 2013;  $S_{CDOM}(400-500) = 0.0179 \text{ nm}^{-1}$ ).

$S_{CDOM}(350-500)$  values for Keszthely and Siófok basins were negatively correlated with  $a_{CDOM}(440)$  (Fig. 3a & b) ( $R^2=0.78$ ,  $p<0.001$  for the Keszthely basin and  $R^2=0.92$ ,  $p<0.001$  for the Siófok basin). The relationship between  $S_{CDOM}$  and  $a_{CDOM}$  in the Szigliget and Szemes basins was also negative ( $R^2=0.91$ ,  $p=0.01$  for Szigliget and  $R^2=0.79$ ,  $p=0.01$  for Szemes) (Fig. 3b).

### 3.1.2. DOC

Seasonal variation in DOC was measured at six permanent sampling stations (Stations 01 and 03 from Basin Keszthely (I), Station 12 from Basin Szigliget (II), Station 20 from Basin Szemes (III), Stations 25 and 30 from Basin Siófok (IV)). DOC concentrations ranged from 7.63 at ST25 in April to 19.70 mg L<sup>-1</sup> at ST01 in July with a mean value of 10.1 mg L<sup>-1</sup> (Table 2). The highest concentrations were observed at ST01 (where the Zala River enters the lake) in summer (July: mean 19.7 mg L<sup>-1</sup>, August: mean 18.6 mg L<sup>-1</sup>) with a slightly smaller secondary peak in early autumn (October: mean 18.9 mg L<sup>-1</sup>) (Fig. 2). However, a slightly different trend was observed in the central part of Keszthely basin at ST03, where DOC started increasing at the end of the summer reaching a maximum in November (12.7 mg L<sup>-1</sup>). For stations furthest from the main inflow, DOC concentrations remained relatively consistent during the course of the year, with two small peaks at ST12 in July and November. DOC concentrations at ST20, 25 and 30 were significantly lower at the  $p < 0.5$  level than ST01, 03 (ANOVA ( $F(1,57) = 31.63$ ,  $p < 0.001$ )).

## 3.2. Spatial variability

### 3.2.1. CDOM optical properties

We observed an  $a_{CDOM}$  gradient across the lake (Fig. 5a) with higher  $a_{CDOM}(440)$  values in Kis-Balaton and the Keszthely basin (I) (where the Zala River enters into the lake) decreasing rapidly towards the northeastern basins near the outflow. The  $a_{CDOM}(440)$  coefficients were markedly different between basins ranging from 0.17 to 7.89 m<sup>-1</sup> with the highest value observed in Keszthely basin at the mouth of Zala River (Fig. 5a and Fig. 6a) (Table 3).  $S_{CDOM}$  coefficients showed marked variability (0.0174-0.0229 nm<sup>-1</sup>) compared to previous studies in inland and marine waters (e.g. Helms et al., 2008). There was no consistent trend in  $S_{CDOM}$  coefficients across the lake although the lowest values generally occurred in the western basin near the inflow (Fig. 5b, Fig. 6b). In Keszthely (I) basin and the western parts of Szigliget (II) basin nearest the inflow of the Zala River, higher variability was observed with lower  $S_{CDOM}$  coefficients more than elsewhere in the lake. In our study,  $S_{CDOM}(350-500)$  exhibited a negative correlation with  $a_{CDOM}(440)$  (Fig. 7a & b) as has been shown in previous studies (e.g., Stedmon et al. 2000, Del Castillo & Coble 2000; Yacobi et al. 2003; Rochelle-Newall et al. 2004; Zhang et al. 2007; Kowalczyk et al. 2003).

The E2:E3 ratio varied significantly ( $R^2=0.47$ ,  $p < 0.001$ ) between the mouth of the river (11.1) and the main outflow (62.0) as specified in Table 3.  $SUVA_{254}$  varied between 2.47 L mg<sup>-1</sup> m<sup>-1</sup> at ST25 in the Siófok basin to 4.45 L mg<sup>-1</sup> m<sup>-1</sup> at ST06 in the Keszthely basin (Table 3). Figure 6d and 6e shows that  $SUVA_{454}$  decreased with

distance from the inflow of the River Zala ( $R^2=0.713$ ,  $p<0.001$ ), whereas E2:E3 ratio increases with increased with distance from the the main inflow.

### 3.2.2. DOC

Concentrations ranged from a minimum of 8.03 at ST17 (Basin III) to 10.9 mg L<sup>-1</sup> at ST07 (Basin 1) with a mean value of 9.66 in the Keszthely basin (I), 8.85 for the Szigliget basin (II), 8.56 for the Szemes basin (III) and 8.66 for the Siófok basin (IV). DOC concentrations slowly decreased with increasing distance from Zala River (Fig. 5c, Fig. 6c), similarly to the trends observed for  $a_{CDOM}(440)$ , but with greater variability through the system than for  $S_{CDOM}$  (coefficient of variation (CV) for  $S_{CDOM} = 0.053$ ) and smaller than for  $a_{CDOM}(440)$  (CV for  $a_{CDOM}(440) = 2.065$ ). DOC concentrations showed a strong and positive relationship with  $a_{CDOM}(440)$  coefficients over the entire dataset (Fig. 8) ( $R^2=0.945$ ,  $p<0.001$ ) although for individual basins this relationship was only significant for Keszthely ( $R^2=0.952$ ,  $p= <0.001$ ).

### 3.3. CDOM photodegradation experiment

Ultraviolet irradiance during the photobleaching experiment ranged from 7.79 to 42.9 MJ m<sup>-2</sup> per day with a mean of 32.4 MJ m<sup>-2</sup> per day (Fig. 9a). The exposure of CDOM to natural solar UV radiation resulted in marked alterations to its absorption properties (Fig. 9).  $a_{CDOM}(440)$  remained relatively constant in the dark control samples (7.04 to 6.18 m<sup>-1</sup>) for the allochthonous treatment from days 0 to 7 (Fig. 9b) indicating minimal bacterial degradation of CDOM. However, the  $CDOM_{allo}$  samples exposed to natural solar radiation demonstrated considerable a reduction in absorption (ANOVA,  $F(1,14) = 12.70$ ,  $p=0.003$ ; Fig. 9b) in the visible part of the spectrum (440 nm) with regard to the dark samples. After 7 days,  $a_{CDOM}(440)$  for the allochthonous samples decreased from 7.04 to 3.36 m<sup>-1</sup>; this equates to a rate of loss of 1.49 m<sup>-1</sup> d<sup>-1</sup> and a 42 % net decrease in absorption from day 0. The difference observed between the control samples and those exposed to natural solar radiation is indicative of a strong photobleaching effect, although it is also possible that bacterial degradation was enhanced in the light. In contrast, there was no discernible, systematic change in the  $CDOM_{auto}$  samples over time. They showed an increase in  $a_{CDOM}(440)$  during the first 3 days (Fig. 9b) from 1.22 to 1.34 m<sup>-1</sup>, but subsequently showed a decrease in absorption from 1.34 to 0.312 m<sup>-1</sup> during the last 4 days, which equates to a 77 % decrease (1.03 m<sup>-1</sup>) from the value at day 3.



The autochthonous control samples ( $CDOM_{\text{auto-dark}}$ ) varied in  $a_{CDOM}(440)$  from 1.22 to 0.596  $m^{-1}$ ; in the absence of photobleaching the decrease in  $a_{CDOM}(440)$  might be explained by residual bacterial activity (although the samples were filtered to minimise bacterial contamination before exposure).

Photodegradation also modified the spectral slope coefficient of the samples (Fig. 9c). The values of  $S_{CDOM}(350-500)$  for the  $CDOM_{\text{allo}}$  samples remained stable, varying less than 0.001  $nm^{-1}$  per day. However, for the  $CDOM_{\text{auto}}$  treatment,  $S_{CDOM}$  coefficients decreased conspicuously until the third day (from 0.009 to 0.005  $nm^{-1}$ ) and then increased with further irradiation from day 3 to day 7 until recovering to its original value (from 0.0051 to 0.0084  $nm^{-1}$ ). Both the spectral slope (ANOVA,  $F(1,14) = 63.20$ ,  $p < 0.001$ ) and absorption coefficient (ANOVA,  $F(1,11) = 208.65$ ,  $p < 0.001$ ) values for  $CDOM_{\text{auto}}$  were significantly lower than those for  $CDOM_{\text{allo}}$ .

Humic-like fluorescence as indicated by  $F_n(355)$  decreased gradually for the  $CDOM_{\text{allo}}$  samples with increasing cumulative UV radiation and exposure time (Fig. 10a) from 41.1 to 17.5 QSU (42.6 % of the original value). Interestingly, no marked changes or clear trend was observed in  $F_n(280)$  (Fig. 10b), suggesting protein-like fluorescence was less susceptible to degradation by natural solar radiation.

There were more than ten orders of magnitude difference in fluorescence intensity between  $CDOM_{\text{allo}}$  and  $CDOM_{\text{auto}}$  samples, presumably driven by the difference in concentration. Given the low concentrations of CDOM, after Milli-Q correction, there was no measurable fluorescence signal for the autochthonous samples.

## 4. Discussion

### 4.1. Dynamics of dissolved organic carbon

CDOM is the coloured fraction of DOC and is often the dominant light absorbing component in lakes, particularly at blue and green wavelengths. Previous research has shown that CDOM can be responsible for up to 80 % of light absorption in Lake Balaton (Riddick et al. 2015) in spite of the fact that the lake also has high concentrations of phytoplankton and non-algal particles (NAP). The high input of DOC from the Zala River results in elevated concentrations in the western basin relative the remainder of the lake (from 0.169  $m^{-1}$  near the outflow to 7.89  $m^{-1}$  at the mouth of the Zala river). High DOC concentrations were largely confined to the waters nearest the inflow but at times the influence of this input could be observed across the westernmost basin and as such must exert influence over metabolic processes in this part of the lake. In comparison to published data from other large systems such as Lake Erie (O'Donnell et al. 2010; 0.19-2.0  $m^{-1}$ ), Lake Champlain (O'Donnell et al. 2013; 0.5-1.15  $m^{-1}$ ) and lakes Peipsi, Vättern and Vänern (Reinart et al. 2004; 0.33-3.82  $m^{-1}$ ) the magnitude of variability in  $a_{CDOM}(440)$  observed in this study was markedly greater (although these previous studies might not have captured

the full range of  $a_{\text{CDOM}}(440)$  variation), which is perhaps surprising given the northerly latitude of some of these lakes (Curtis 1998). However, the magnitude variability in  $a_{\text{CDOM}}(440)$  observed in this study is not too dissimilar from that reported previously in other systems such as Lake Taihu (Zhang et al. 2011; 1.37 to 9.55 m<sup>-1</sup>).

The seasonal pattern in CDOM absorption and DOC concentration varied considerably in the western basin, but was relatively constant in other basins. The annual peak(s) in  $a_{\text{CDOM}}(440)$  and DOC occurred in spring and/or autumn some stations (e.g., ST03, ST12, ST30) were broadly coincident with or lagged slightly behind the annual runoff maxima suggesting a seasonal trend that was partly driven by the flushing of organic matter from catchment soils during high flow events. This pattern is common in many temperate and boreal lakes where DOC export from catchments is driven by the availability of flushable terrestrial carbon sources and the seasonality of precipitation and/or snowmelt.

Conversely, at the station nearest to the inflow of the River Zala the main peak in  $a_{\text{CDOM}}(440)$  and DOC occurred in summer (August and July respectively) with a smaller secondary peak in DOC the autumn. The summer peak in  $a_{\text{CDOM}}(440)$  and DOC at the inflow of the River Zala was clearly related to the proximity of the station to the mouth of the River Zala and a high input of humic-rich water from the Kis-Balaton wetland complex. In wetlands, high production of DOC can occur during the growing season due to leaching from plants and biological degradation of organic detritus (Pinney et al. 2000; Freeman et al. 2004). In our study system, this summer peak in DOC production also coincides with the annual rainfall minimum and the period of lowest flow into the lake, resulting in a concentrated input of humic-rich water and elevated CDOM absorption at the inflow of the Zala and across the western basin.

It is also notable that the effect of this humic-rich water from the River Zala on the biogeochemistry and light climate in Lake Balaton diminishes rapidly through the system in summer. This can be partly attributed to the dilution of the inflow with less humic water, but also the rapid degradation of the highly biologically and photochemically reactive DOC originating from Kis-Balaton during a period when microbial activity is high due to warm water temperatures and UV irradiance is at its maximum. The collective residence time of the Keszthely and Szigliget basins (0.25 and 0.72 years respectively; Somlyódy & van Straten 2012) explains why the highly humic and labile components of the DOC entering the lake from the River Zala are largely degraded before reaching the Szemes basin with only the most recalcitrant DOC fractions persisting beyond the westernmost basins. The resulting differences in CDOM composition are clearly reflected in the variability in the CDOM absorption characteristics ( $S_{\text{CDOM}}$ ,  $\text{SUVA}_{254}$  and  $\text{E2:E3}$ ) observed through the system. These findings agree with

other field and experimental studies which have shown that CDOM can be rapidly degraded by photobleaching in summer (Vodacek et al. 1997; Del Vecchio & Blough 2002; Zhang, et al. 2009).

The mean (and range) in  $S_{CDOM}(350-500)$  for Lake Balaton in summer was  $0.0211 \text{ nm}^{-1}$  ( $0.0174$  to  $0.0229 \text{ nm}^{-1}$ ), higher than values reported for several other systems including Lake Erie (Binding et al. 2008;  $S_{CDOM}(350-400)$   $0.0163 \text{ nm}^{-1}$ ) and Lake Chapman (O'Donnell et al. 2013;  $S_{CDOM}(400-500) = 0.0179 \text{ nm}^{-1}$ ) (Fig 2 & Fig. 6 b). The values reported by Binding et al. (2008 and O'Donnell et al. (2013) were more comparable to the mean slopes observed for the humic-rich waters encountered in Kis-Balaton ( $0.0189 \text{ nm}^{-1}$ ) and the Keszthely basin ( $0.0199 \text{ nm}^{-1}$ ). However, it should be stressed that some of this variation could in part be explained by the different wavelength ranges used in the calculation of  $S_{CDOM}$ . The magnitude of spatial variability in Lake Balaton was more comparable to that reported for northern Lake Taihu, where  $S_{CDOM}$  was found to vary between  $0.0127$  to  $0.0190$ , from  $0.0159$  to  $0.0220$  and from  $0.0122$  to  $0.0174 \text{ nm}^{-1}$  for the wavelength domains  $280$  to  $500 \text{ nm}$ ,  $280$  to  $360 \text{ nm}$  and  $360$  to  $400 \text{ nm}$  respectively (Zhang et al. 2007) and between  $0.0180$  to  $0.0281 \text{ nm}^{-1}$  (for  $S_{CDOM}(280-500)$ ) in Zhang et al. (2011).

Very few studies have investigated seasonal variation in  $S_{CDOM}$  in lakes. In the present study, seasonal variation in  $S_{CDOM}(350-500)$  was greatest in the eastern basin furthest from the inflow ranging from  $0.0158 \text{ nm}^{-1}$  to  $0.0300 \text{ nm}^{-1}$  (Table 2, Fig. 2) with a mean annual value of  $0.0205 \text{ nm}^{-1}$ . The range observed in Lake Balaton was greater than previously reported in other lake systems. Ylöstalo et al. (2014) for instance reported a mean (range) of  $0.0182 \text{ nm}^{-1}$  ( $0.0155$ - $0.0200 \text{ nm}^{-1}$ ) for 15 boreal lakes in Southern Finland within the summer months. Interestingly, there is more variation in  $S_{CDOM}$  in lakes and other optically complex inland waters (Kowalczyk et al. 2003; Zhang et al. 2007) than shelf sea environments despite the fact the latter are globally diverse and far more extensive (Babin et al. 2003).

The structural modifications in DOM and its coloured fractions that are in part conveyed through variation in  $S_{CDOM}$  result from interplay between the input of allochthonous DOC from the catchment, the production of autochthonous DOC from the microbial digestion of phytoplankton cells and the rate at which these materials are degraded biologically and photochemically (Zhang, et al. 2009; Yamashita & Tanoue 2004; Vantrepotte et al. 2007; Nelson et al. 1998; Yamashita et al. 2013). Newly produced autochthonous CDOM typically has a higher  $S_{CDOM}$  coefficient compared to humic-rich allochthonous material (Bracchini et al. 2010). Photobleaching also results in an increase in  $S_{CDOM}$ . The majority of sampling stations in Lake Balaton exhibited higher slope coefficients during the summer months, which could be attributed to an increase in new autochthonous DOC

production from the growth and decay of phytoplankton during the summer bloom period and intense  
440 photobleaching of humic-rich material received from the catchment. Seasonal variability in  $S_{CDOM}$  was notably  
lower at the inflow of the River Zala than in the easternmost basins, which again probably reflects the marked  
effect that intense summer photobleaching has on the structural composition of dissolved organic matter as it slow  
moves through the system.

Noticeably, while  $a_{CDOM}(440)$  and  $S_{CDOM}(350-500)$  demonstrated a strong inverse relationship over the entire  
445 dataset, the slope of this relationship varied significantly between the different basins in the lake. The slope of the  
relationship highest in the eastern basin and lower in the west near the inflow where DOC concentrations were  
the highest. The relationship between  $a_{CDOM}(440)$  and  $S_{CDOM}$  is known to be influenced by both its provenance  
and any subsequent transformations (Carder et al. 1989; Helms et al. 2008) that take place. The observed trends  
in Lake Balaton are likely to be a result of the mixing of water rich in allochthonous carbon from the River Zala  
450 with more dilute and autochthonous carbon sources in the main lake and the progressive degradation of this  
material via photobleaching as it moves through the system. Comparable trends have been found by Zhang et al.  
(2007) in the Yunnan Plateau lakes.

E2:E3 ratio variation found in this study have also been shown in previous studies (Santos et al. 2014; Helms  
1998). In coastal waters E2:E3 values are typically within the range  $8.70 \pm 1.4$  and  $13.5 \pm 1.6$  (nearshore – offshore  
455 in Georgia Bight; Helms et al. 2008). In inland and transitional waters, E2:E3 ratios as high as 14.6 have been  
reported in Lake Taihu (Zhang et al. 2011) and up to 26.9 in Chesapeake Bay (Helms et al. 2008). E2:E3 values  
for Lake Balaton were significantly higher and more variable than previously reported varying between 11.0 and  
72.0, with the highest values near the outflow indicating the CDOM here was less humified and had a lower  
molecular weight. Increasing values of E2:E3 ratio have been reported by other authors indicating a decrease in  
460 colour as well as in molecular weight (Helms et al. 2008; Peuravuori & Pihlaja 1997).

Similar trends were also observed in  $SUVA_{254}$ . In Lake Balaton,  $SUVA_{254}$  varied between  $2.5 \text{ mg}^{-1} \text{ m}^{-1}$  in the  
easternmost basin and  $4.5 \text{ L mg}^{-1} \text{ m}^{-1}$  near the mouth of the River Zala. This again indicates that water entering  
from the river contained high molecular weight dissolved organic carbon with a high content of aromatic  
substances (Weishaar et al. 2003), whereas the compounds comprising the DOC in the central and eastern parts  
465 of the lake had a lower molecular size and aromaticity. The  $SUVA_{254}$  values measured in Lake Balaton were  
broadly comparable to those reported for other lake systems. For example, Song et al. (2013) reported a maximum  
value of  $8.7 \pm 2.8 (\text{L mg}^{-1} \text{ m}^{-1})$  for 26 inland water bodies in China.  $SUVA_{254}$  values were lower than those

reported for marine waters where the relative contribution of autochthonous carbon sources is often greater. The sensitivity of  $SUVA_{254}$  to changes in the carbon provenance is shown by Asmala et al. (2013) who obtained a range  $SUVA_{254}$  values of  $3.58 \pm 0.33$   $5.41 \pm 0.40$   $L\ mg^{-1}\ m^{-1}$  in three Baltic Sea estuaries whereas measurements taken from stations on the sea shelf varied between  $1.87 \pm 0.09$  and  $3.47 \pm 0.27$   $L\ mg^{-1}\ m^{-1}$ .

## 4.2. CDOM photobleaching

The spatio-seasonal variability in CDOM absorption in Lake Balaton strongly suggests that photobleaching plays a key role in the processing and degradation of dissolved organic carbon as it flows through the system. Rapid degradation of allochthonous CDOM was observed (Fig. 9), which was especially pronounced at the time of year with the highest solar radiation but probably also enhanced by mineralisation by bacterial activity as a response to high water temperatures during the summer period. Dilution processes alone cannot explain the loss of DOC; therefore, it must also be due to in-lake transformation. The processing and transformation of DOC by photobleaching not only influences carbon cycling, but it also is accompanied by an increase in the transparency of the water column (Osburn et al. 2009) and changes in the optical properties (Yamashita et al. 2013) that have wider implications for the underwater light climate and primary production.

The in-lake incubations conducted in Lake Balaton provide further substantiation for the critical role of photochemistry in the turnover of CDOM. We observed rapid changes in the absorption properties of CDOM in response to exposure to natural UV irradiation. In the allochthonous CDOM treatments, the rate of degradation resulted higher than that obtained for Lake Taihu by Zhang et al. (2013) who reported a 22 % decrease over 9 days. Bacterial degradation was not noticeable in the allochthonous samples as there was almost no difference in  $a_{CDOM}(440)$  for the dark treatment compared to the initial value (Fig. 9d) although we cannot exclude the possibility of enhanced bacterial degradation in light exposed treatments (Kragh et al. 2008).

Photodegradation also modified the spectral slope (Fig. 9b) of the CDOM absorption spectra. Both the spectral slope and absorption coefficient for autochthonous CDOM were significantly lower than for allochthonous samples (ANOVA,  $F(1,27) = 6.55$ ,  $p=0.01$ ). During our experiment,  $S_{CDOM}(350-500)$  did not follow a systematic trend in the allochthonous samples, varying less than  $0.0007\ m^{-1}$  per day. However, for the autochthonous treatment, it decreased conspicuously until the third day (from  $0.0087$  to  $0.0051\ m^{-1}$ ) but then increased again from day 3 to day 7 until it almost returned to its original value (from  $0.0051$  to  $0.0084\ m^{-1}$ ). The increase in slope matches with an increase in  $a_{CDOM}(440)$  and has been considered by Helms et al. (2008) to be a result of transformation from high to low molecular weight CDOM and is considered to be a response to photo-induced

decomposition (Moran et al. 2000; Grzybowski 2000; Yamashita et al. 2013). The initial decrease in slope during the early part of the experiment echoes observations by Yamashita et al. (2013) and Fichot & Benner (2012) whom attributed this phenomena to microbial degradation of bioavailable CDOM (Nelson et al. 2004).

The fluorescence spectra also indicate a marked difference in composition between the allochthonous and autochthonous material. The decomposition of CDOM into lower molecular weight compounds under UV-B radiation (Lepane et al. 2003) results in a significant loss of both absorption and fluorescence. The negligible fluorescence signal observed for the autochthonous CDOM samples in this study is likely due to its low concentration. In contrast, the humic-like fluorescence signal measured from allochthonous samples was initially high but decreased over the experimental period from 41.07 to 17.48 QSU (57.5 % decrease). Similarly, we observed a reduction in protein-like fluorescence from 2.06 to 1.93 QSU (6.31 % decrease; Fig. 10b) over the 7 days of the experiment. This agrees strongly with the results of previous studies showing the fluorescence signal from humic compounds is rapidly lost through photobleaching, whereas aromatic-like fluorescence is generally not as susceptible to photo-degradation. Helms et al. (2013) for example reported an 84% decrease in humic-like fluorescence in response to photobleaching compared to an only 47 % decrease in aromatic-like fluorescence after 68 days of continuous irradiation in a UV solar simulator.

#### 4.3. Implications for the underwater light field

The absorption of light by CDOM is a major determinant of water transparency in lakes and the availability of light for primary production (Kirk 1994; Laurion et al. 1997, 2000; Vähätalo et al. 2005). Absence of measurements of the underwater light field makes it difficult to attribute its effect to this particular case, but there clearly exists evidence that the dynamic nature of dissolved organic carbon in lakes results in marked spatio-seasonal variation in both the magnitude and wavelength-dependency of light absorption by chromophoric substances. This variability undoubtedly has implications not only for the quantity of light available to photosynthetic organisms but also its quality. High concentrations of CDOM result in intense absorption of light at blue and green wavelengths but the intensity of absorption decreases exponentially with wavelength. This not only has implications for the productivity of the system (Cory et al. 2015), but also for the photo-physiology and species composition of the phytoplankton community. The intense absorption of UV light by CDOM protects phytoplankton from physiological damage and reduces the need for phytoplankton cells to manufacture UV-protective pigments. This can result in chromatic acclimation with phytoplankton in high CDOM waters investing less in UV-protective pigments (Riddick et al. 2015).

The magnitude of variability in the spectral dependency of CDOM absorption also has implications for bio-optical models of the underwater light field that are used to underpin remote sensing algorithms for estimation of CDOM in lakes and other inland waters. Existing bio-optical models (Lee et al. 2002) commonly extrapolate absorption by CDOM in the blue to longer wavelengths using a fixed slope coefficient. We demonstrate here that even within a single lake system significant variability can occur in  $S_{CDOM}$ . Failure to accommodate variability in  $S_{CDOM}$  in bio-optical models will lead to errors not only in the estimation of CDOM absorption but also in the contributions of other optically-active substances (e.g., chlorophyll, non-algal particles) to light absorption and scattering within the water column. In Lake Balaton, the variability observed in  $S_{CDOM}$  ( $0.0174 - 0.0289 \text{ nm}^{-1}$ ) could produce errors up to 180 % and 900 % on estimates of  $a_{CDOM}$  at wavelengths in the blue (350 nm) and red (650 nm) respectively. This suggests that new approaches are needed to incorporate knowledge on the variability in  $S_{CDOM}$  into adaptive bio-optical modelling frameworks for optically-complex waters to improve our ability to model the underwater light field and increase the performance of physics-based remote sensing algorithms for CDOM retrieval.

## 5. Conclusions

This study revealed the high spatial and seasonal variability in the quantity and quality of CDOM that can exist within a large, temperate shallow lake. The variation was strongly driven by the allochthonous input of dissolved carbon from the Zala River and its rapid transformation as it moves through the system. The variability in the quantity and quality of CDOM was strongly reflected in a number of readily measured optical parameters including  $S_{CDOM}$ , E2:E3 ratio and  $SUVA_{254}$ , which collectively pointed towards a marked decrease in the molecular weight of dissolved carbon compounds, a reduction in its aromatic content and a decrease in the degree of humification as water moved through the system from the main inflow to outflow.

Photobleaching was found to be a major factor controlling the in-lake transformation and degradation of CDOM, and a key process influencing the spatial structure CDOM throughout the system. The photobleaching rate coefficient for allochthonous CDOM was found to be higher than for autochthonous CDOM due to the greater photoreactivity of terrestrially-derived compounds. CDOM in Lake Balaton is mainly terrestrial in origin and is thus rapidly degraded by exposure UV irradiation. The implied importance of photobleaching to carbon dynamics is consistent with previous studies conducted in other inland water bodies (Zhang et al. 2013) as well as other studies carried out in shelf seas (Babin et al. 2003) and the open ocean (Helms et al. 2013).

More widely, these results provide an insight on the potential contribution of wetlands to DOM and CDOM in lakes, not only in terms of the concentration of CDOM but also its seasonality. The seasonal trend in CDOM observed close to the main inflow was significantly different from that observed elsewhere in the system.

Notwithstanding the fact that most of the CDOM in Lake Balaton would seem to be terrestrial in origin, we did observe an increase in  $a_{\text{CDOM}}(440)$  in autumn following the breakdown of phytoplankton blooms on the lake.

The observed spatial and temporal variability in the optical properties of CDOM in this study has important implications for biogeochemical cycling in Lake Balaton but also for bio-optical models of the underwater light climate in lakes and their application in the parametrization of algorithms for optical remote sensing of CDOM and other optically-active constituents.

## 6. Data availability

The data analysed in this study are accessible through the University of Stirling DataSTORRE Open Access Repository at <http://hdl.handle.net/1893/24009>.

### *Author contributions*

M.E. Aulló-Maestro designed and conducted the experiments with input from P. Hunter, E. Spyarakos and A. Tyler. Hajnalka Horváth and Mátyás Présing carried out data collection for seasonal measurements at Balaton Limnological Institute. Spatial measurements were taken by GloboLakes and INFORM teams. Jesús M. Torres Palenzuela contributed to fluorescence measurements at University of Vigo and Tom Preston contributed to mass-spectrometry analysis at the Stable Isotope Biochemistry Laboratory at the Scottish Universities Environmental Research Centre (SUERC). M.E. Aulló-Maestro processed the data and prepared the figures with input from Pierre Mercatoris. M.E. Aulló-Maestro prepared the manuscript with the assistance of all co-authors.

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## Tables

835 **Table 1. Seasonal variability of  $a_{CDOM}$  (440) for permanent stations**

Month	ST01	ST02	ST03	ST04	ST05	ST06
3	3.694	1.435	NA	NA	0.265	NA
4	5.485	1.244	0.617	0.468	0.228	0.302
5	6.831	0.912	0.603	0.615	0.221	0.329
6	6.872	0.637	0.417	0.152	0.145	0.062
7	7.547	0.670	0.419	0.274	0.088	0.111
8	9.005	0.746	0.507	0.431	0.258	0.189
9	6.172	0.930	0.633	0.265	0.196	0.170
MAX	9.005	1.435	0.633	0.615	0.265	0.329
MIN	3.694	0.637	0.417	0.152	0.088	0.062
MEAN	6.515	0.939	0.533	0.367	0.200	0.194

**Table 2. Values of CDOM absorption coefficient at 440nm, CDOM slope coefficient between 350 and 500nm, DOC concentration, E2:E3 ratio, SUVA254 and mean distance of the basin to River Zala. Values obtained for CDOM seasonal variation.**

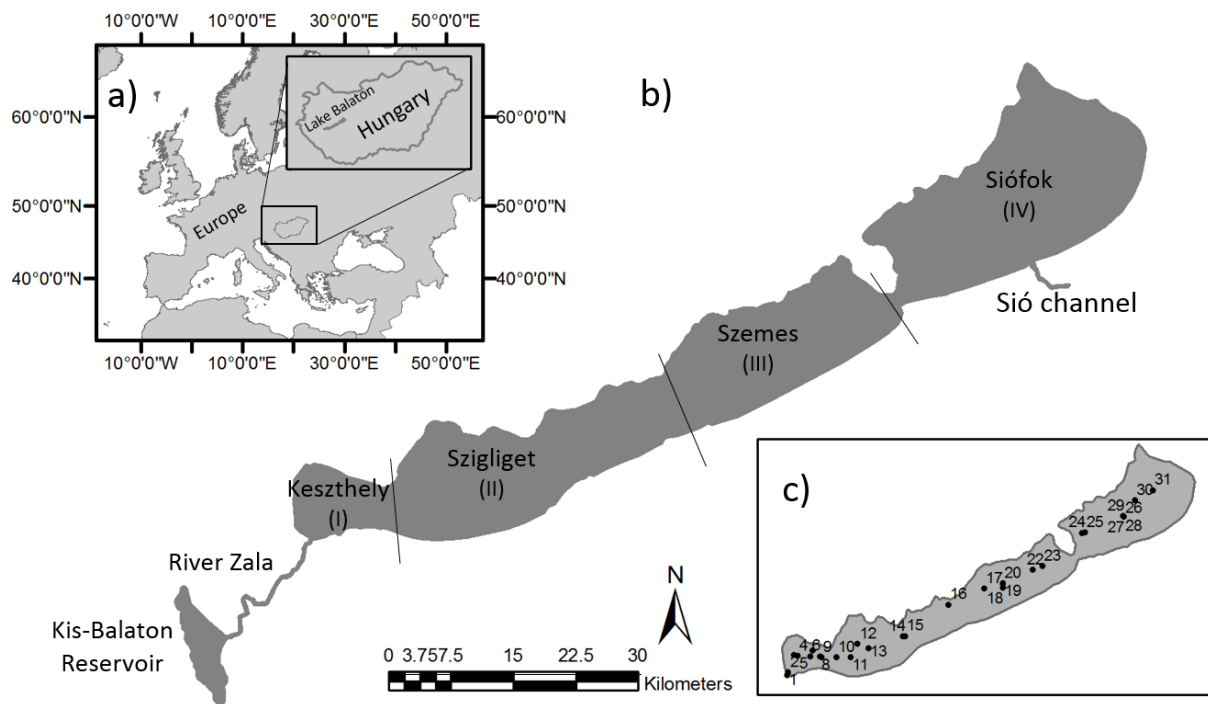
Station	Basin	$a_{\text{CDOM}}(440) (\text{m}^{-1})$	$S_{\text{CDOM}}(350-500) (\text{nm}^{-1})$	$[\text{DOC}] \text{ mg} \cdot \text{L}^{-1}$	Distance to River Zala (Km)
		Min - Max Season Season (Mean±SD)	Min - Max Season Season (Mean±SD)	Min - Max Season Season (Mean±SD)	
01	Keszthely	3.69 - 9.01 Summer - Spring (6.52±1.54)	0.0161 - 0.0181 Spring - Summer (0.0173±0.0006)	10.02 - 19.70 Summer - Winter (16.08±2.88)	0.48
03		0.64 - 1.43 Spring - Summer (0.94±0.28)	0.0187 - 0.0221 Summer - Summer (0.0201±0.0011)	8.97 - 12.67 Autumn - Spring (10.15±1.04)	3.59
12	Szigliget	0.42 - 0.63 Autumn - Summer (0.53±0.09)	0.0185 - 0.0230 Summer - Summer (0.0211±0.0016)	8.88 - 10.85 Autumn - Spring (9.51±0.66)	14.95
20	Szemes	0.15 - 0.61 Spring - Summer (0.37±0.15)	0.0175 - 0.0275 Summer - Summer (0.0213±0.0035)	8.23 - 9.00 Summer - Autumn (8.76±0.23)	39.73
25	Siófok	0.09 - 0.26 Spring - Summer (0.20±0.06)	0.0185 - 0.0279 Summer - Summer (0.0215±0.0033)	7.63 - 8.54 Summer - Spring (8.13±0.25)	62.86
30		0.06 - 0.33	0.0158 - 0.0300	7.82 - 8.36	69.16

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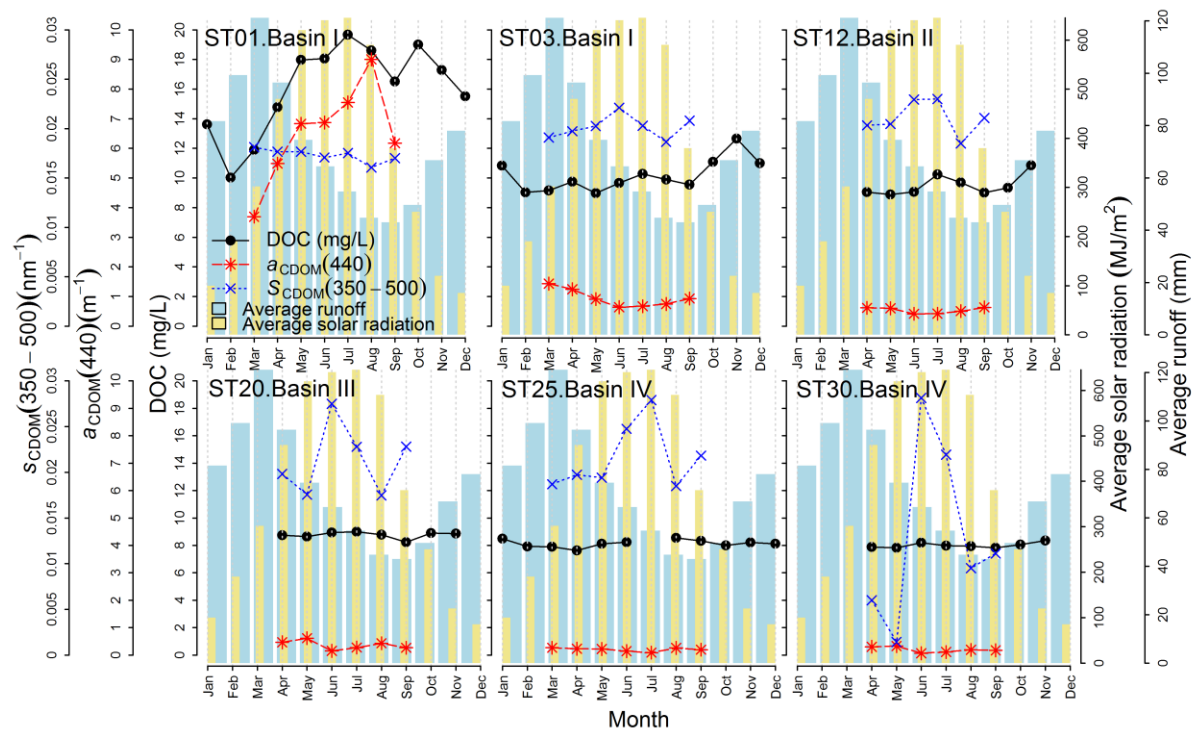
Spring - Summer	Summer - Spring	Autumn - Spring
<b>(0.19±0.10)</b>	<b>(0.0219±0.0049)</b>	<b>(8.01±0.18)</b>

**Table 3. Values of CDOM absorption coefficient at 440nm, CDOM slope coefficient between 350 and 500nm, DOC concentration, E2:E3 ratio, SUVA<sub>254</sub> and mean distance of the basin to River Zala. Values obtained for CDOM spatial variation (values July 2014).**

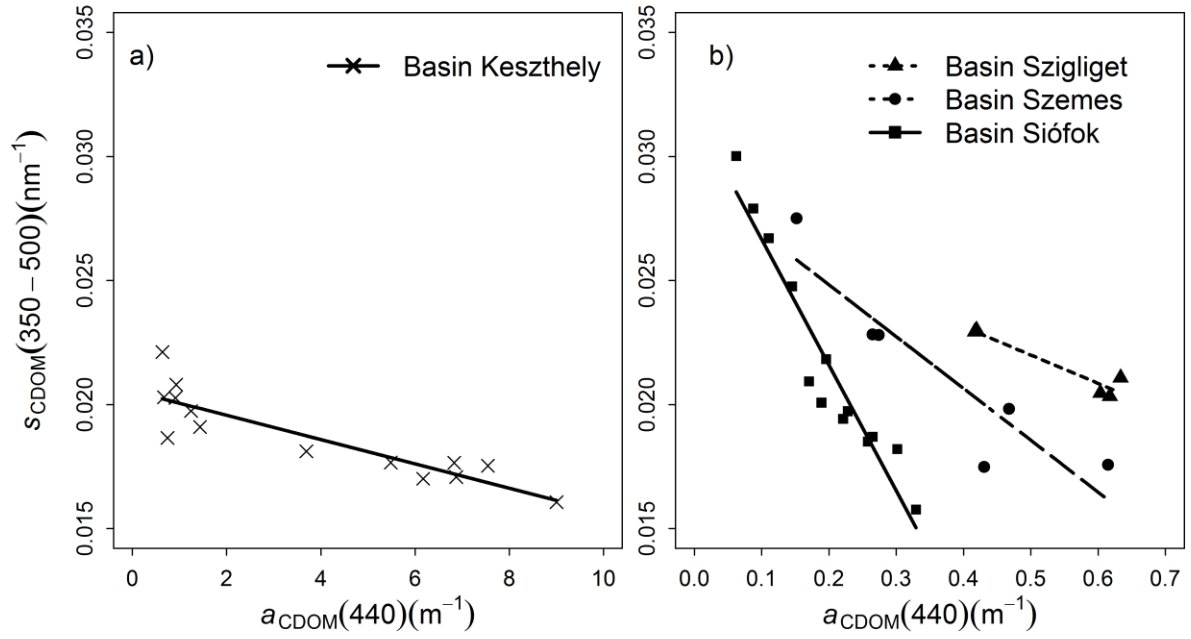
Basin	$a_{\text{CDOM}}(440) \text{ (m}^{-1}\text{)}$ Min - Max (Mean)	$S_{\text{CDOM}}(350-500) \text{ (nm}^{-1}\text{)}$ Min - Max (Mean)	[DOC] $\text{mg} \cdot \text{L}^{-1}$ Min - Max (Mean)	E2:E3 ratio Min - Max (Mean)	SUVA <sub>254</sub> ( $\text{L} \cdot \text{mg}^{-1} \cdot \text{m}^{-1}$ ) Min - Max (Mean)	Mean distance to River Zala (Km)
Kis-Balaton	2.45 - 4.66 (3.58)	0.0186 - 0.0191 (0.0189)	---	---	---	
Kestzthely	0.57 - 7.89 (1.49)	0.0174 - 0.0212 (0.0199)	10.9 - 8.85 (9.66)	34.1 - 11.1 (15.9)	4.45 - 3.59 (4.04)	3.84
Szigliget	0.33 - 4.31 (1.00)	0.0190 - 0.0214 (0.0209)	9.63 - 8.50 (8.85)	28.8 - 14.4 (18.9)	4.35 - 3.24 (3.70)	17.4
Szemes	0.26 - 0.34 (0.294)	0.0211 - 0.0221 (0.0215)	9.07 - 8.03 (8.56)	44.3 - 18.4 (27.2)	3.82 - 2.62 (3.12)	40.0
Siófok	0.17 - 0.21 (0.193)	0.0203 - 0.0229 (0.0215)	8.99 - 8.14 (8.66)	62.0 - 24.1 (42.2)	3.13 - 2.47 (2.69)	60.5



**Figure 1. a) Location of Lake Balaton within Europe. b) Map of basins, Kis-Balaton Reservoir, River Zala and Sió Channel. c) Location of 31 sampling stations in Lake Balaton**

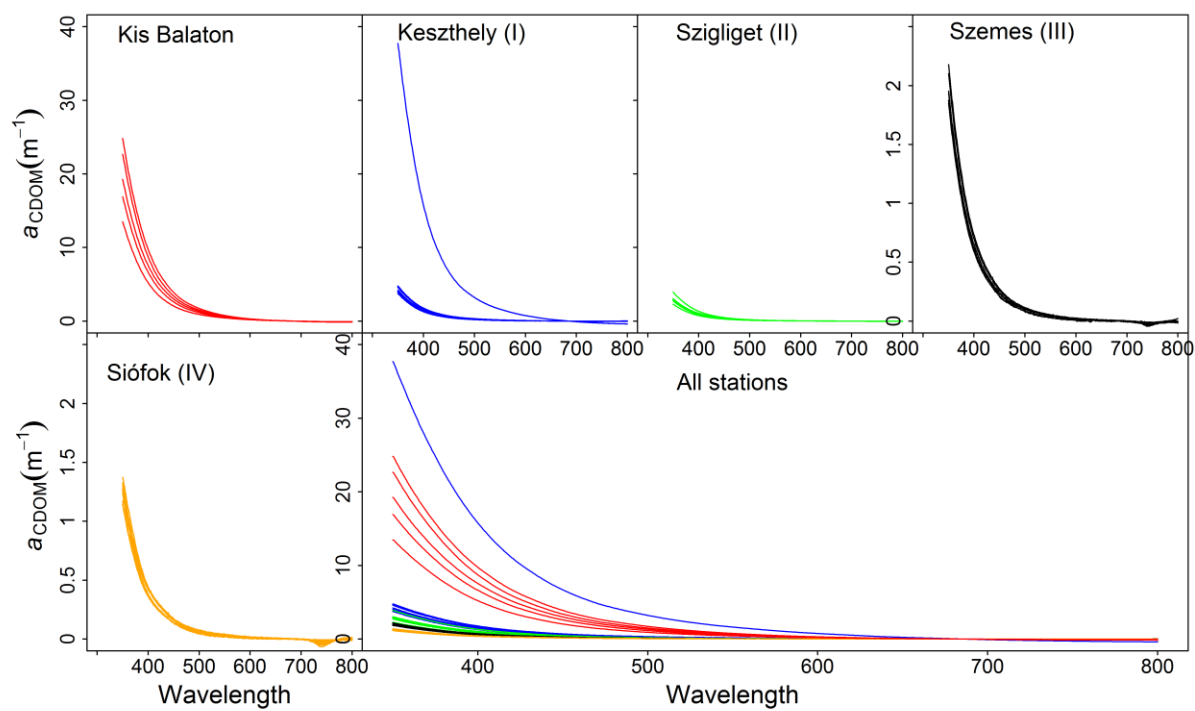


**Fig. 2** Seasonal  $a_{CDOM}(440)$ ,  $S_{CDOM}(350-500)$  and DOC concentration variation in Lake Balaton between January and December 2014 and seasonal variability of runoff in Balaton region (Hungary), monthly means from 1921 to 2007 (modified from Anda & Varga, 2010).

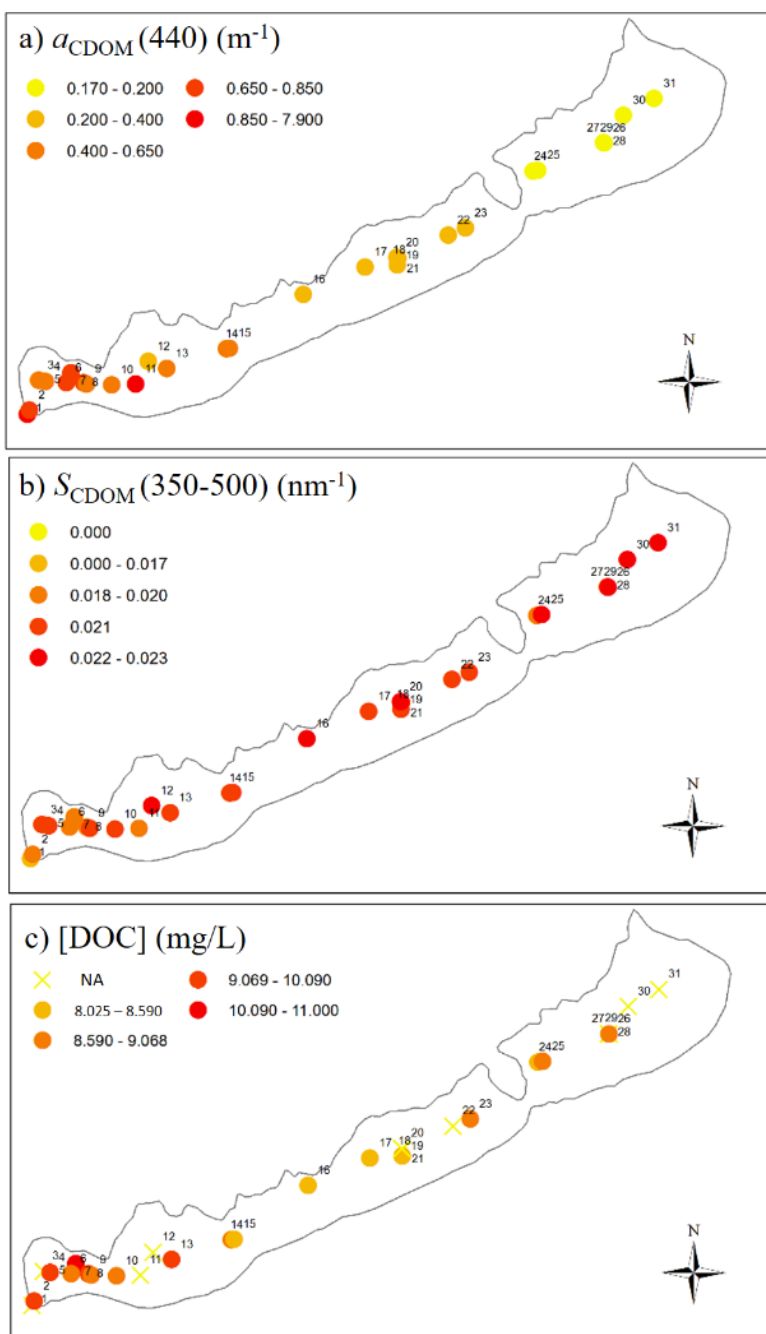


**Fig. 3. Plot of  $S_{CDOM}(350-500)$  as a function of  $a_{CDOM}(440)$  using the seasonal sampling data for (a) basin Keszthely,  $S_{CDOM}(350-500) = -0.0005 \cdot a_{CDOM}(440) + 0.0205$ ,  $R^2=0.7833$ ,  $p<0.001$  and (b) basin Szigliget,  $S_{CDOM}(350-500) = -0.0114 \cdot a_{CDOM}(440) + 0.0277$ ,  $R^2=0.9122$ ,  $p=0.011$ ; basin Szemes,  $S_{CDOM}(350-500) = -0.0209 \cdot a_{CDOM}(440) + 0.0209$ ,  $R^2=0.7932$ ,  $p=0.0108$  and basin Siófok,  $S_{CDOM}(350-500) = -0.0507 \cdot a_{CDOM}(440) + 0.0317$ ,  $R^2=0.9154$ ,  $p<0.001$ .**

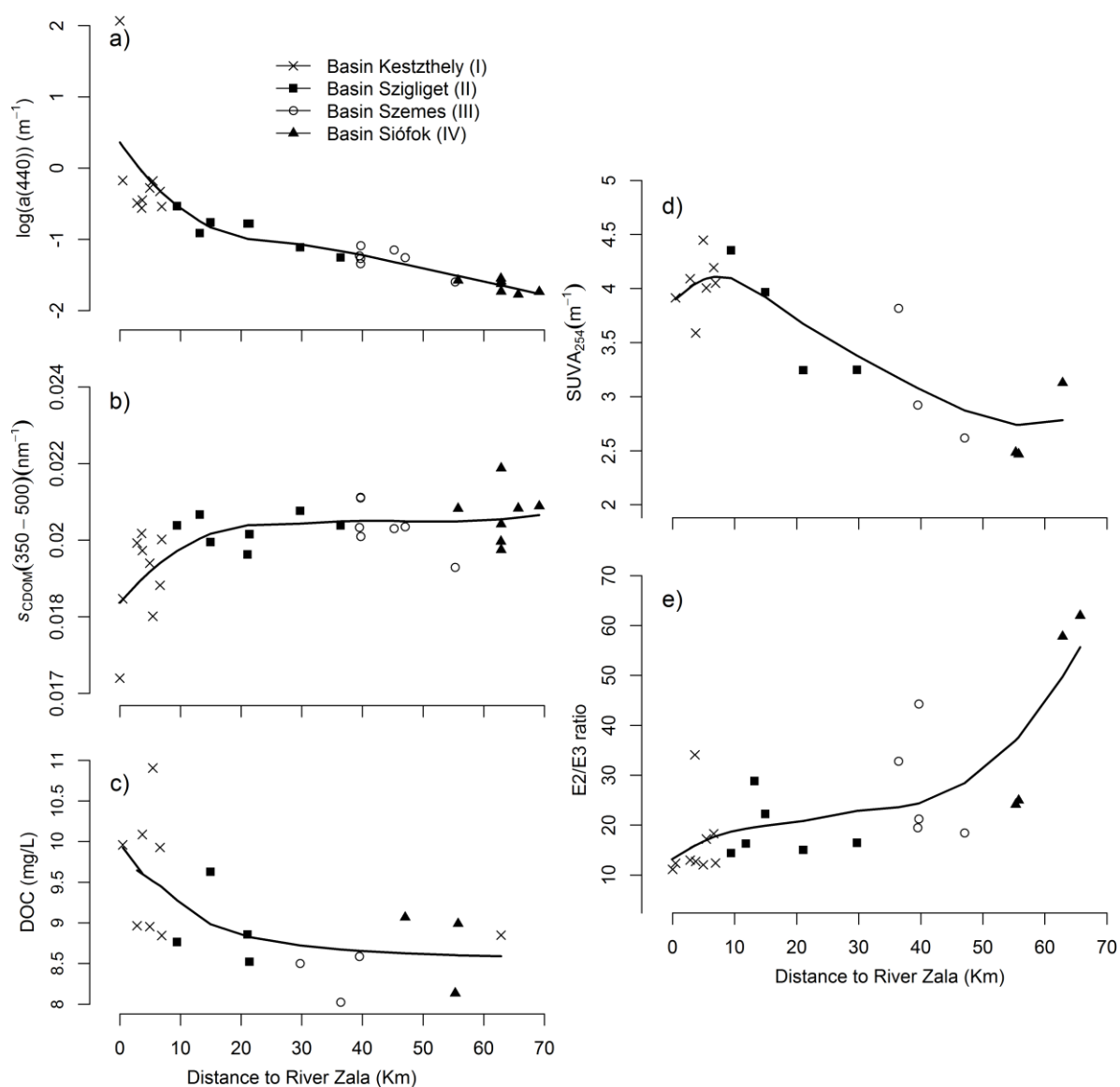




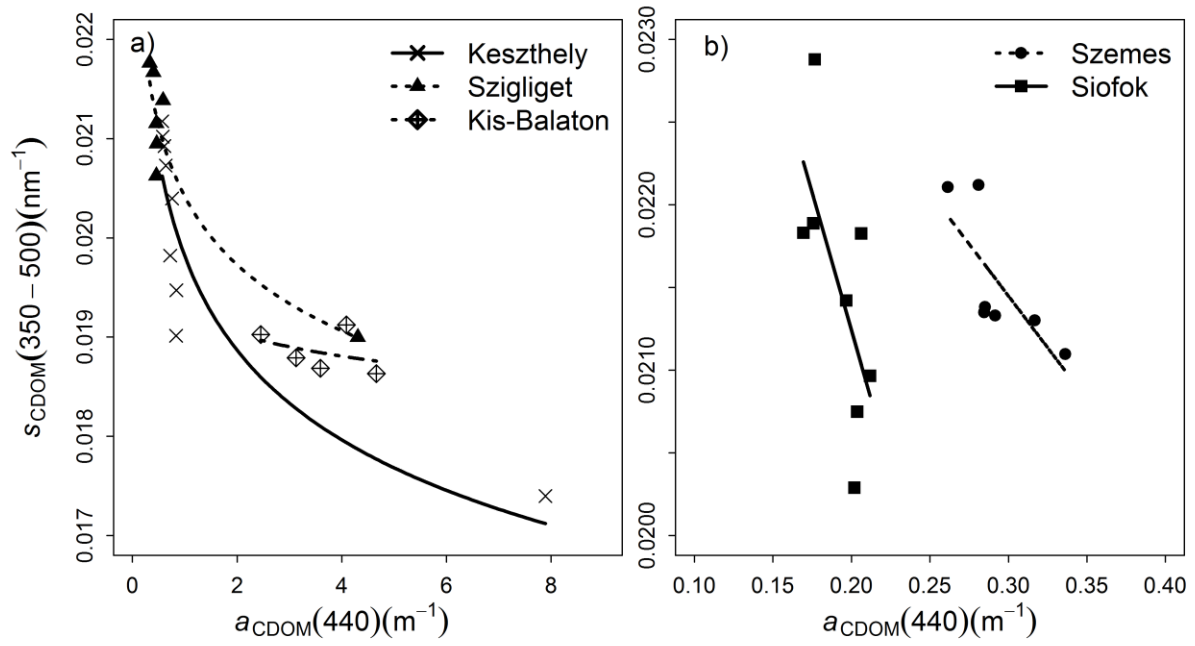
**Fig. 4** CDOM absorption spectra for all stations (per basin) and Kis-Balaton. Note the different y-axis scale for the Szemes and Siófok basins.



**Figure 5. a) Spatial  $a_{\text{CDOM}}(440)$  variation in Lake Balaton per station. b) Spatial  $S_{\text{CDOM}}(350-500)$  variation in Lake Balaton per station. c) Spatial DOC concentration in Lake Balaton per station. Data derived from measurements made in July 2014.**

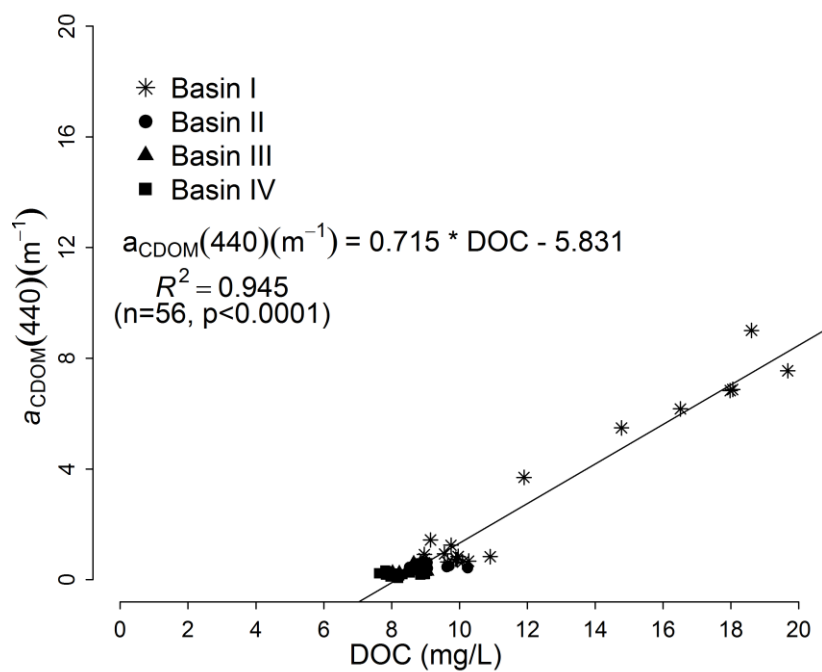


**Figure 6. Scatterplots against distance to the main inflow [Km] with loess curve fitted to data. (a) Variation of CDOM absorption coefficient at 440 nm ( $a_{CDOM}(440)$ ) [ $m^{-1}$ ], (b) CDOM slope coefficient between 350 and 500 nm ( $S_{CDOM}(350-500)$ ) [ $nm^{-1}$ ] variation, (c) DOC concentration [mg/L] variation, (d) specific UV absorptivity at 254nm ( $SUVA_{254}$ ) [ $m^{-1}$ ] and e) E2/E3 ratio as a function of distance from the Zala River during the summer 2014 campaign.**



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**Figure 7. Plot of  $S_{CDOM}(350-500)$  as a function of  $a_{CDOM}(440)$  spatial variation. a) Kis Balaton,  $S_{CDOM}(350-500) = 0.019266 \cdot a_{CDOM}(440)^{-0.017362}$ ; basin Keszthely,  $S_{CDOM}(350-500) = 0.019817 \cdot a_{CDOM}(440)^{-0.070820}$  and basin Szigliget,  $S_{CDOM}(350-500) = 0.020418 \cdot a_{CDOM}(440)^{-0.070820}$ . b) Basins Szemes,  $S_{CDOM}(350-500) = -0.01252 \cdot a_{CDOM}(440) + 0.02521$  and Siofok,  $S_{CDOM}(350-500) = -0.03330 \cdot a_{CDOM}(440) + 0.027900$ .**



**Figure 8.** Scatterplot of  $a_{CDOM}(440)$  plotted as a function of DOC concentration (mg/L). Line is a regression curve by least squares fit.

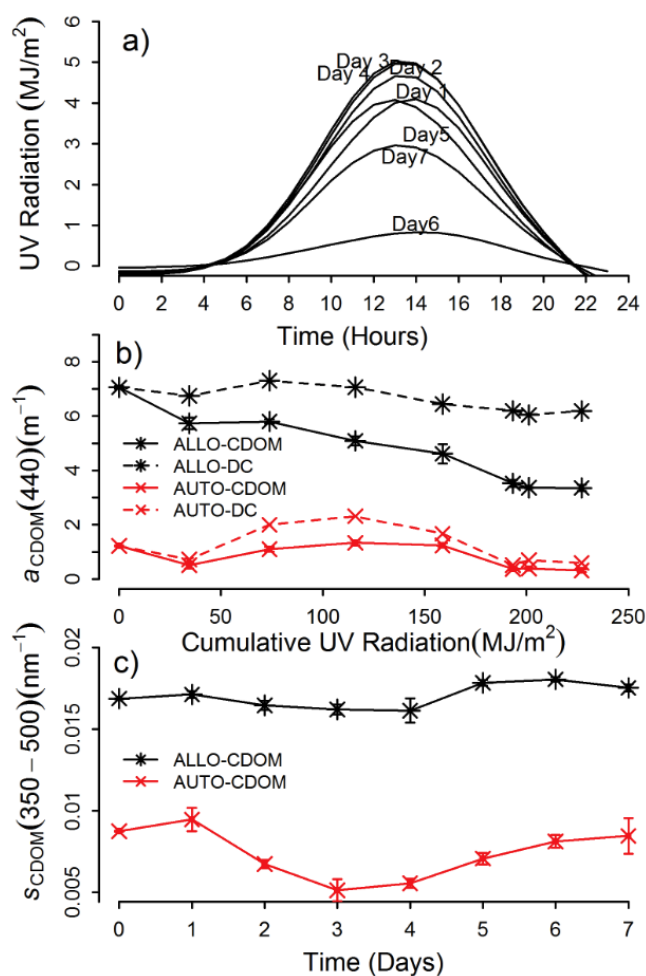
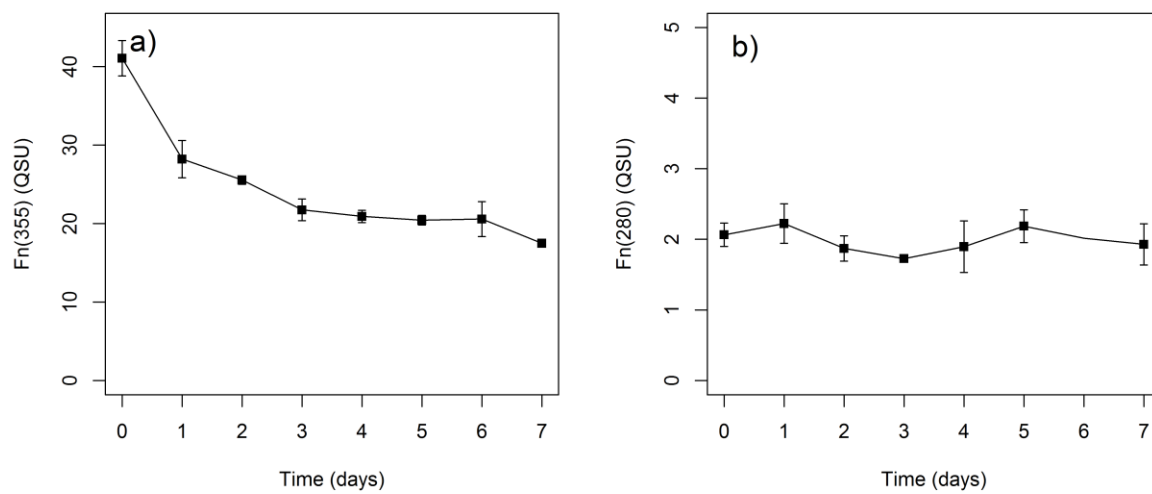


Figure 9. a) Ultraviolet irradiance during the photobleaching experiment. b) Variation of  $S_{\text{CDOM}}(350-500)$  per day. c) Variation of  $a_{\text{CDOM}}(400)$  accumulated UV radiation. d) Variation of  $a_{\text{CDOM}}(400)$  per day. Note that the error bars represent  $\pm$  standard deviation and exist for every data point corresponding with exposed samples in sub-figures b), c) and d), dark samples not included.



**Figure 10. Changes in humic-like fluorescence ( $F_n(355)$ ) and protein-like fluorescence ( $F_n(280)$ ) for allochthonous CDOM samples with time during photobleaching experiment. Bars =  $\pm$ Standard Deviation.**

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