**Supplementary Material**
Dissolved Organic Matter in the Gulf of Cádiz: Distribution and Drivers of Chromophoric and Fluorescent Properties

Valentina Amaral*, Cristina Romera-Castillo, Jesús Forja

*Correspondence: vamaral@cure.edu.uy

1. Supplementary Figures

Fig. S1. Description of the water masses in the Gulf of Cádiz, (A) T-S diagram and (B) Proportion of the water masses determined by the OMP analysis. Data corresponds to the deepest station (SP7) and Sancti Petri section during summer of 2016, respectively. SAW: Superficial Atlantic Water, NACW: North Atlantic Central Water and MOW: Mediterranean Outflow Water.
Fig. S2. Fluorescence characteristics of the six components identified by the PARAFAC global analysis.
Fig. S3. Excitation (solid line) and emission (dotted line) spectra of the six-components for the global model and the same components for the individual models (components were numbered arbitrarily by the PARAFAC models) for summer (C1S - C6S), autumn (C1A - C6A), spring and winter (C1Sp-W - C3Sp-W). The * denotes comparisons between components with a Tucker congruence values < 0.98.
Fig. S4. Vertical profiles of temperature (°C), salinity, apparent oxygen utilization (AOU, µM), chlorophyll a (Chl a, µg L⁻¹) and dissolved organic carbon (DOC, µM) from Guadalquivir transect during the stratified (spring) and mixed (autumn) period.
Fig. S5. Vertical profiles of fluorescence components C1-C6 during the stratified (spring) and mixed (autumn) period in the Guadalquivir transect.
Fig. S6. Relationship between average seasonal values of UV radiation (UVA+UVB) and fluorescent intensity of component 4 (C4).

Fig. S7. Absorbance spectra for station SP7 during summer and winter at different depths: black 300 m, red 500 m and green 650 m.
2. Supplementary Tables

Table S1. Complementary information about oceanographic and meteorological settings in the Gulf of Cádiz during the study period. * Correspond to the accumulated precipitations 30 days prior to the sampling dates.

| Variable                     | March     | June      | September | December  |
|------------------------------|-----------|-----------|-----------|-----------|
| Thermocline depth (m)        | 75.4 ± 42.3 | 43.9 ± 16.8 | 34.8 ± 14.4 | 67.3 ± 26.9 |
| Accumulative precipitations  | 40.2      | 74.9      | 8.8       | 59.2      |
| (L m⁻²)*                     |           |           |           |           |
| UV radiation (W m⁻²)         | 247.7 ± 3.3 | 466.8 ± 10.2 | 338.7 ± 14.8 | 166.9 ± 12.4 |
| Wind speed (m s⁻¹)           | 8.4 ± 4.3 | 7.8 ± 3.8 | 5.9 ± 2.9 | 8.0 ± 4.1 |
Table S2. Spectral properties of the six PARAFAC components from the global model in the Gulf of Cádiz (n = 766) and correspondence with previously identified components in different environments using OpenFluor database and literature*.

| Ex/Em     | OpenFluor (Tucker congruency) | Ex/Em     | Description                                      | Environment                                      |
|-----------|-------------------------------|-----------|--------------------------------------------------|-------------------------------------------------|
| C1 270 (320)/411 | C2, (Chen et al., 2018) (0.99) | 260 (305)/404 | Marine humic-like                                | Arctic Sea water                                 |
|          | C2, (Murphy et al., 2006) (0.98) | 320/414   | Marine humic-like                                | Atlantic and Pacific Ocean                       |
|          | C3, (Yamashita et al., 2010b)(0.98) | > 260 (> 315)/421 | Microbial humic-like                             | Tropical rivers                                  |
|          | C2, (Catalá et al., 2015)(0.98) | 270 (326)/402 | In situ, Microbial                               | Global dark ocean                                |
|          | C1, (Yamashita et al., 2011)(0.97) | 260 (320)/425 | Humic-like enriched in fulvic acids              | Watersheds                                       |
|          | C415, (Kida et al., 2019)(0.97) | -         | Ubiquitous, autochthonous and allochthonous     | Antarctic lakes                                  |
| C1 260 (310)/429 | C1, (Garcia et al., 2015) (0.97) | 240 (310)/429 | Peak A+M, terrestrial                            | Mountain streams                                 |
| C2 260 (320)/425 | C2, (Dalmagro et al., 2019)(0.97) | 252 (321)/408 | Terrestrial                                      | Streams riparian forest                          |
| C2 318/410 | C2, (Li et al., 2016) (0.97) | 260 (305)/416 | Terrestrial (humic and fulvic)                   | Everglades                                       |
|          | C3, (Yamashita et al., 2010c)(0.97) | -         | Ubiquitous                                       | Pantanal                                         |
| C1 252 (310)/398 | C1, (Murphy et al., 2018) (0.97) | 318/410   | Marine/microbial                                 | Artic porewater                                  |
|          | C2, (Chen et al., 2016) (0.97) | 260 (311)/411 | Peak A                                           | Mesocosm experiment                              |
|          | C2, (Asmala et al., 2018) (0.96) | 240 (340)/398 | Peak A                                           | Coastal zone                                     |
| C1 240/308 | C1, (Kulkarni et al., 2019)(0.96) | 240/308   | Terrestrial                                      | Groundwater                                      |
|          | C1, (Borisover et al., 2009) (0.96) | 240 (310)/396 | Marine humic-like                                | Lake                                            |
| Region                  | Sample | Reference                  | Method                | Type                                | Source of Wastewater |
|-------------------------|--------|----------------------------|-----------------------|-------------------------------------|----------------------|
| Marine and terrestrial, peak M. Humic-like, terrestrial | Pacific and Atlantic Ocean Groundwater |
| C2                      | 300/359 | C4, (Schittich et al., 2018) (0.97) | 300/350 | Protein like | Groundwater |
|                         |        | C3, (Catalá et al., 2015) (0.97) | 298/343 | Tryptophan-like | Global dark ocean |
|                         |        | C3, (Amaral et al., 2016) (0.96) | 300/340 | Tryptophan-like | Coastal subtropical lagoon Sediments |
|                         |        | C4, (Chen et al., 2017) 0.96 | 300/338 | Bound proteins | Recycled wastewaters |
|                         |        | C6, (Murphy et al., 2011) (0.96) | 290/352 | Protein, Tryptophan-like | |
| C3                      | 275-370/446 | C3, (Chen et al., 2016) (0.97) | 275 (370)/ 452 | Terrestrial humic-like | Artic porewater |
|                         |        | C3, (Graeber et al., 2012) 0.97 | 255 (370)/432 | Humic-like, ubiquitous | Central European streams |
|                         |        | C1, (Yamashita et al., 2010a)(0.96) | 260 (370)/466 | Mix A/C, higher plant derived and microbial reworked | Okhtoks Sea and the North Pacific Ocean |
| C4                      | 250/430 | C2, (Walker et al., 2009) (0.98) | < 240/404 | Humic-like, terrestrially, exposed to UVA | Canadian Arctic surface water Lakes |
|                         |        | C3, (Osburn et al., 2011) (0.98) | < 250/434 | Terrestrial, Peak A | Tropical rivers, Africa Sediments Streams |
|                         |        | C4, (Lambert et al., 2016a) (0.98) | <260/444 | Terrestrial and photochemically degraded | |
|                         |        | C2, (Chen et al., 2017) (0.98) | <260/434 | Terrestrial, photoprodut and/or photorefractory | |
|                         |        | C4, (Lambert et al., 2016b)(0.98) | <240/434 | Terrestrial and photochemical degradation | |
|                         |        | C3, (Osburn et al., 2017) (0.98) | 240(305)/425 | Humic substances after degradation | Lakes |
|                         |        | C4, (Murphy et al., 2008) (0.97) | 250(320)/370 | Unknown, anthropogenic | Pacific and Atlantic Ocean |
| ID  | Authors and Year (Reference) | 265/422 | Description                                                                 | Location                                |
|-----|------------------------------|---------|------------------------------------------------------------------------------|-----------------------------------------|
| C1  | Chen et al., 2016 (0.97)     | 97      | Terrestrial                                                                 | Artic Ocean porewater                  |
| C1  | Cawley et al., 2012 (0.97)   | 97      | Terrestrial, Peak A                                                           | Gulf of Maine                           |
| C3  | Osburn et al., 2016a (0.96)  | 97      | Terrestrial humic-like                                                        | Coastal waters                          |
| C2  | Kowalczyk et al., 2009 (0.96)| 97      | Terrestrial humic-like                                                        | South Atlantic Bight                    |
| C3  | Li et al., 2016 (0.96)       | 97      | Peak A                                                                       | Lakes                                   |

| ID  | Authors and Year (Reference) | 275/325 (349) | Description                                                                 | Location                                |
|-----|------------------------------|--------------|------------------------------------------------------------------------------|-----------------------------------------|
| C5  | 280/317 (378)                | 92           | Bound to proteins                                                            | Atlantic Ocean                          |
| C3  | Kowalczyk et al., 2013 (0.92)| 92           | Mixture of PAH and aromatic amino acids                                      | Italian coast                            |
| C1  | Gonnelli et al., 2016*       | 92           | Peak N, PAH, origin unclear                                                  | Black Sea                                |
| C3  | Margolin et al., 2018*       | 92           | Peak T, leaching of polyphenols from senescence plants                        | Headwater catchments                    |
| C2  | Garcia et al., 2018 (0.92)   | 92           | Similar to free dissolved tryptophan                                          | Marine, streams, wastewaters            |
| C4  | Asmala et al., 2018 (0.91)   | 91           | Oil-related                                                                  | Estuaries                               |

| ID  | Authors and Year (Reference) | 275/320 | Description                                                                 | Location                                |
|-----|------------------------------|---------|------------------------------------------------------------------------------|-----------------------------------------|
| C6  | 275/303                      | 93      | Protein-like                                                                 | Atlantic and Pacific Ocean              |
| C5  | Osburn et al., 2016b(0.91)   | 91      | Tyrosine                                                                     | Atlantic coastal plain                  |
Table S3. Parameters of the multiple linear regressions model between dissolved organic carbon (DOC), absorption coefficient $a_{254}$ and fluorescent components with temperature (T) and salinity (S) for surface water. Fitting parameter of the relationship with T ($\beta_1$) and S ($\beta_2$), standard error and the determination coefficient ($R^2$) are presented. The significance levels of the estimation are included ($p < 0.0001 ***$, $p < 0.001 **$ and $p < 0.01 *$). Results are described for each campaign and only cases with p-values higher than 0.01 are described.

| Component | Season | $\beta_1$ (T) | $\beta_2$ (S) | Std. Error | $R^2$ |
|-----------|--------|---------------|---------------|------------|-------|
| DOC       | Spring | -3.2*         | 41.4***       | 11.9       | 0.09  |
|           | Summer | 1.9***        | -30.0***      | 8.9        | 0.28  |
|           | Autumn | 2.2***        | -9.2          | 6.2        | 0.36  |
|           | Winter | 5.7***        | -32.8***      | 5.5        | 0.22  |

| $a_{254}$  | Season | $\beta_1$ (T) | $\beta_2$ (S) | Std. Error | $R^2$ |
|------------|--------|---------------|---------------|------------|-------|
| DOC        | Spring | -0.643***     | 4.085***      | 0.301      | 0.16  |
| Winter     | -0.0005 *** | -0.0092*** | 0.0016       | 0.69      |
| Autumn     | 0.085*** | -0.870**     | 0.185         | 0.34      |
| Winter     | 0.164*** | -2.070***    | 0.211         | 0.30      |

| C1         | Season | $\beta_1$ (T) | $\beta_2$ (S) | Std. Error | $R^2$ |
|------------|--------|---------------|---------------|------------|-------|
| DOC        | Spring | -0.0011 **    | -0.0011       | 0.0011     | 0.50  |
| Summer     | 0.0005 *** | -0.0092***  | 0.0016       | 0.69      |
| Winter     | 0.0011 *** | -0.0344***  | 0.0017       | 0.79      |

| C2         | Season | $\beta_1$ (T) | $\beta_2$ (S) | Std. Error | $R^2$ |
|------------|--------|---------------|---------------|------------|-------|
| DOC        | Spring | -0.0011*      | 0.0040        | 0.0009     | 0.24  |
| Winter     | -0.0001 | -0.0025*     | 0.0005       | 0.31      |

| C4         | Season | $\beta_1$ (T) | $\beta_2$ (S) | Std. Error | $R^2$ |
|------------|--------|---------------|---------------|------------|-------|
| DOC        | Spring | -0.0019       | 0.0204**      | 0.0022     | 0.27  |

| C5         | Season | $\beta_1$ (T) | $\beta_2$ (S) | Std. Error | $R^2$ |
|------------|--------|---------------|---------------|------------|-------|
| DOC        | Spring | -0.0082***    | 0.0461***     | 0.0030     | 0.28  |
| Summer     | 0.0006* | -0.0199***   | 0.0043       | 0.36      |
| Winter     | 0.0004 | -0.0137**    | 0.0031       | 0.16      |

| C6         | Season | $\beta_1$ (T) | $\beta_2$ (S) | Std. Error | $R^2$ |
|------------|--------|---------------|---------------|------------|-------|
| DOC        | Spring | -0.0183***    | 0.1034***     | 0.0071     | 0.26  |
| Summer     | 0.0015** | -0.0235***  | 0.0100       | 0.13      |
References

Amaral, V., Graebner, D., Calliari, D., and Alonso, C. (2016). Strong linkages between DOM optical properties and main clades of aquatic bacteria. Limnol. Oceanogr. 61, 906–918. doi:10.1002/lno.10258.

Asmala, E., Haraguchi, I., Markager, S., Massicotte, P., Riemann, B., Staehr, P. A., et al. (2018). Eutrophication Leads to Accumulation of Recalcitrant Autochthonous Organic Matter in Coastal Environment. Global Biogeochem. Cycles 32, 1673–1687. doi:10.1002/2017GB005848.

Borisover, M., Laor, Y., Parparov, A., Bukhanovsky, N., and Lado, M. (2009). Spatial and seasonal patterns of fluorescent organic matter in Lake Kinneret (Sea of Galilee) and its catchment basin. Water Res. 43, 3104–3116. doi:10.1016/j.watres.2009.04.039.

Catalá, T. S., Reche, I., Fuentes-Lema, A., Romero-Castillo, C., Nieto-Cid, M., Ortega-Retuerta, E., et al. (2015). Turnover time of fluorescent dissolved organic matter in the dark global ocean. Nat. Commun. 6, 1–8. doi:10.1038/ncomms6986.

Cawley, K. M., Butler, K. D., Aiken, G. R., Larsen, L. G., Huntington, T. G., and McKnight, D. M. (2012). Identifying fluorescent pulp mill effluent in the Gulf of Maine and its watershed. Mar. Pollut. Bull. 64, 1678–1687. doi:10.1016/j.marpolbul.2012.05.040.

Chen, M., Jung, J., Lee, Y. K., and Hur, J. (2018). Surface accumulation of low molecular weight dissolved organic matter in surface waters and horizontal off-shelf spreading of nutrients and humic-like fluorescence in the Chukchi Sea of the Arctic Ocean. Sci. Total Environ. 639, 624–632. doi:10.1016/j.scitotenv.2018.05.205.

Chen, M., Kim, J. H., Nam, S. I., Niessen, F., Hong, W. L., Kang, M. H., et al. (2016). Production of fluorescent dissolved organic matter in Arctic Ocean sediments. Sci. Rep. 6, 1–10. doi:10.1038/srep39213.

Chen, M., Kim, S. H., Jung, H. J., Hyun, J. H., Choi, J. H., Lee, H. J., et al. (2017). Dynamics of dissolved organic matter in riverine sediments affected by weir impoundments: Production, benthic flux, and environmental implications. Water Res. 121, 150–161. doi:10.1016/j.watres.2017.05.022.

D’Sa, E. J., Overton, E. B., Lohrenz, S. E., Maiti, K., Turner, R. E., and Freeman, A. (2016). Changing Dynamics of Dissolved Organic Matter Fluorescence in the Northern Gulf of Mexico Following the Deepwater Horizon Oil Spill. Environ. Sci. Technol. 50, 4940–4950. doi:10.1021/acs.est.5b04924.

Dalmagro, H. J., Lathuilhière, M. J., Sallo, F. da S., Guerreiro, M. F., Pinto, O. B., de Arruda, P. H. Z., et al. (2019). Streams with riparian forest buffers versus impoundments differ in discharge and DOM characteristics for pasture catchments in Southern Amazonia. Water (Switzerland) 11, 1–20. doi:10.3390/w11020390.

Garcia, R. D., Diéguez, M. del C., Gerea, M., Garcia, P. E., and Reissig, M. (2018). Characterisation and reactivity continuum of dissolved organic matter in forested headwater catchments of Andean Patagonia. Freshw. Biol. 63, 1049–1062. doi:10.1111/fwb.13114.

Garcia, R. D., Reissig, M., Queimaldiños, C. P., Garcia, P. E., and Dieguez, M. C. (2015). Climate-driven terrestrial inputs in ultraoligotrophic mountain streams of Andean Patagonia revealed through chromophoric and fluorescent dissolved organic matter. Sci. Total Environ. 521–522, 280–292. doi:10.1016/j.scitotenv.2015.03.102.

Gonçalves-Araujo, R., Stedmon, C. A., Heim, B., Dubinenvok, I., Kraberg, A., Moiseev, D., et al. (2015). From fresh to marine waters: Characterization and fate of dissolved organic matter in the Lena River Delta Region, Siberia. Front. Mar. Sci. 2, 1–13. doi:10.3389/fmars.2015.00108.

Gonnelli, M., Galletti, Y., Marchetti, E., Mercadante, L., Retelletti Brogi, S., Ribotti, A., et al. (2016). Dissolved organic matter dynamics in surface waters affected by oil spill pollution: Results from the Serious Game exercise. Deep. Res. Part II Top. Stud. Oceanogr. 133, 88–99. doi:10.1016/j.dsr2.2015.06.027.

Graeber, D., Gelbrecht, J., Pusch, M. T., Anlanger, C., and von Schiller, D. (2012). Agriculture has changed the amount and composition of dissolved organic matter in Central European headwater streams. Sci. Total Environ. 438, 435–446. doi:10.1016/j.scitotenv.2012.08.087.

Kida, M., Kojima, T., Tanabe, Y., Hayashi, K., Kudoh, S., Maie, N., et al. (2019). Origin, distributions, and environmental significance of ubiquitous humic-like fluorophores in Antarctic lakes and streams. Water Res. 163, 0–2. doi:10.1016/j.watres.2019.114901.

Kowalczyk, P., Durako, M. J., Young, H., Kahn, A. E., Cooper, W. J., and Gonsior, M. (2009). Characterization of dissolved organic matter fluorescence in the South Atlantic Bight with use of PARAFAC model: Interannual variability. Mar. Chem. 113, 182–196. doi:10.1016/j.marchem.2009.01.015.

Kowalczyk, P., Tilstone, G. H., Zablocka, M., Rötgers, R., and Thomas, R. (2013). Composition of dissolved organic matter along an Atlantic Meridional Transect from fluorescence spectroscopy and
Kulkarni, H., Mladenov, N., and Datta, S. (2019). Effects of acidification on the optical properties of dissolved organic matter from high and low arsenic groundwater and surface water. *Sci. Total Environ.* 653, 1326–1332. doi:10.1016/j.scitotenv.2018.11.040.

Kulkarni, H. V., Mladenov, N., Johannesson, K. H., and Datta, S. (2017). Contrasting dissolved organic matter quality in groundwater in Holocene and Pleistocene aquifers and implications for influencing arsenic mobility. *Appl. Geochemistry* 77, 194–205. doi:10.1016/j.apgeochem.2016.06.002.

Lambert, T., Bouillon, S., Darchambeau, F., Massicotte, P., and Borges, A. V. (2016a). Shift in the chemical composition of dissolved organic matter in the Congo River network. *Biogeoosciences* 13, 5405–5420. doi:10.5194/bg-13-5405-2016.

Lambert, T., Teodoru, C. R., Nyoni, F. C., Bouillon, S., Darchambeau, F., Massicotte, P., et al. (2016b). Along-stream transport and transformation of dissolved organic matter in a large tropical river. *Biogeoosciences* 13, 2727–2741. doi:10.5194/bg-13-2727-2016.

Li, P., Lee, S. H., Lee, S. H., Lee, J. B., Lee, Y. K., Shin, H. S., et al. (2016). Seasonal and storm-driven changes in chemical composition of dissolved organic matter: a case study of a reservoir and its forested tributaries. *Environ. Sci. Pollut. Res.* 23, 24834–24845. doi:10.1007/s11356-016-7720-z.

Margolin, A. R., Gonnelli, M., Hansell, D. A., and Santinelli, C. (2018). Black Sea dissolved organic matter dynamics: Insights from optical analyses. *Limnol. Oceanogr.* 63, 1425–1443. doi:10.1002/lno.10791.

Murphy, K. R., Hambly, A., Singh, S., Henderson, R. K., Baker, A., Stuetz, R., et al. (2011). Organic matter fluorescence in municipal water recycling schemes: Toward a unified PARAFAC model. *Environ. Sci. Technol.* 45, 2909–2916. doi:10.1021/es103015e.

Murphy, K. R., Ruiz, G. M., Dunsmaur, W. T. M., and Waite, T. D. (2006). Optimized parameters for fluorescence-based verification of ballast water exchange by ships. *Environ. Sci. Technol.* 40, 2357–2362. doi:10.1021/es051935e.

Murphy, K. R., Stedmon, C. A., Waite, T. D., and Ruiz, G. M. (2008). Distinguishing between terrestrial and autochthonous organic matter sources in marine environments using fluorescence spectroscopy. *Mar. Chem.* 108, 40–58. doi:10.1016/j.marchem.2007.10.003.

Murphy, K. R., Stedmon, C. A., Wenig, P., and Bro, R. (2014). OpenFluor- An online spectral library of auto-fluorescence by organic compounds in the environment. *Anal. Methods* 6, 658–661. doi:10.1039/c3ay41935e.

Murphy, K. R., Timko, S. A., Gonsior, M., Powers, L. C., Wünsch, U. J., and Stedmon, C. A. (2018). Photochemistry Illuminates Ubiquitous Organic Matter Fluorescence Spectra. *Environ. Sci. Technol.* 52, 11243–11250. doi:10.1021/acs.est.8b02648.

Osburn, C. L., Anderson, N. J., Stedmon, C. A., Giles, M. E., Whiteford, E. J., McGenity, T. J., et al. (2017). Shifts in the Source and Composition of Dissolved Organic Matter in Southwest Greenland Lakes Along a Regional Hydro-climatic Gradient. *J. Geophys. Res. Biogeosciences* 122, 3431–3445. doi:10.1002/2017JG003999.

Osburn, C. L., Boyd, T. J., Montgomery, M. T., Bianchi, T. S., Coffin, R. B., and Paerl, H. W. (2016a). Optical proxies for terrestrial dissolved organic matter in estuaries and coastal waters. *Front. Mar. Sci.* 2. doi:10.3389/fmars.2015.00127.

Osburn, C. L., Handsel, L. T., Peterls, B. L., and Paerl, H. W. (2016b). Predicting Sources of Dissolved Organic Nitrogen to an Estuary from an Agro-Urban Coastal Watershed. *Environ. Sci. Technol.* 50, 8473–8484. doi:10.1021/acs.est.6b00053.

Osburn, C. L., Wigdahl, C. R., Fritz, S. C., and Saros, J. E. (2011). Dissolved organic matter composition and photoactivity in prairie lakes of the U.S. Great Plains. *Limnol. Oceanogr.* 56, 2371–2390. doi:10.4319/lo.2011.56.6.2371.

Schittich, A. R., Wünsch, U. J., Kulkarni, H. V., Battistel, M., Bregnhoj, H., Stedmon, C. A., et al. (2018). Investigating Fluorescent Organic-Matter Composition as a Key Predictor for Arsenic Mobility in Groundwater Aquifers. *Environ. Sci. Technol.* 52, 13027–13036. doi:10.1021/acs.est.8b04070.

Stedmon, C. A., and Markager, S. A. (2005). Tracing the Production and Degradation of Autochthonous Fractions of Dissolved Organic Matter by Fluorescence Analysis. *Limnol. Oceanogr.* 50, 1415. Available at: http://search.ebscohost.com/login.aspx?direct=true&db=edsjsrt&AN=edsjsr.3597686&authtype=sso &custid=s8993828&site=eds-live&scope=site.

Walker, S. A., Amon, R. M. W., Stedmon, C., Duan, S., and Louchoourn, P. (2009). The use of PARAFAC modeling to trace terrestrial dissolved organic matter and fingerprint water masses in coastal Canadian Arctic surface waters. *J. Geophys. Res. Biogeosciences* 114, 1–12.
