Turnover time of fluorescent dissolved organic matter in the dark global ocean

Catalá, Teresa Serrano; Reche, Isabel; Fuentes-Lema, Antonio; Romera-Castillo, Cristina; Nieto-Cid, Mar; Ortega-Retuerta, Eva; Calvo, Eva; Alvarez, Marta; Marrase, Celia; Stedmon, Colin; Alvarez-Salgado, X. Anton

Published in:
Nature Communications

Link to article, DOI:
1038/ncomms6986

Publication date:
2015

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):
Turnover time of fluorescent dissolved organic matter in the dark global ocean

Teresa S. Catalá, Isabel Reche, Antonio Fuentes-Lema, Cristina Romera-Castillo, Mar Nieto-Cid, Eva Ortega-Retuerta, Eva Calvo, Marta Álvarez, Cèlia Marrasé, Colin A. Stedmon & X. Antón Álvarez-Salgado

Marine dissolved organic matter (DOM) is one of the largest reservoirs of reduced carbon on Earth. In the dark ocean (>200 m), most of this carbon is refractory DOM. This refractory DOM, largely produced during microbial mineralization of organic matter, includes humic-like substances generated in situ and detectable by fluorescence spectroscopy. Here we show two ubiquitous humic-like fluorophores with turnover times of 435 ± 41 and 610 ± 55 years, which persist significantly longer than the ~350 years that the dark global ocean takes to renew. In parallel, decay of a tyrosine-like fluorophore with a turnover time of 379 ± 103 years is also detected. We propose the use of DOM fluorescence to study the cycling of resistant DOM that is preserved at centennial timescales and could represent a mechanism of carbon sequestration (humic-like fraction) and the decaying DOM injected into the dark global ocean, where it decreases at centennial timescales (tyrosine-like fraction).
**Global distribution of fluorescent components.** The maximum fluorescence intensity (Fmax) of the two humic-like components (C1 and C2) obtained during the Malaspina circumnavigation showed a global distribution similar to the apparent oxygen utilization (AOU; Fig. 3a–c), reaching their maxima in the Eastern North Pacific central and intermediate waters and their minima in the Indian Ocean central waters. In contrast, this global trend with AOU was not evident for the two amino-acid-like components (C3 and C4; Fig. 3d,e).

**Relationships between the archetypical AOU and fluorophores.** Combining the outputs from the water mass, AOU and fluorescence analyses, we calculated WT proportion-weighted average values, hereafter referred to as archetypal, for the AOU and the fluorescence intensities of the four components (see Methods). Archetypal values retain the variability associated with the initial concentrations at the site where each WT is defined and its transformation by basin-scale mineralization processes up to the study site12. Archetypal concentrations explained 81, 77, 75, 24 and 26% of the total variability of AOU, C1, C2, C3 and C4, respectively (Table 1).

In Fig. 4, we show the measured maximum fluorescence intensity (grey dots), archetypal values for each WT (white dots) and archetypal values for each sample (black dots) for the components C1, C2, C3 and C4 (Fig. 4a–d, respectively). We obtained direct relationships between the archetypal humic-like components (C1 and C2) and archetypal AOU (Fig. 4a,b) suggesting a net production of these components in parallel with the water mass ageing. The relatively high archetypal fluorescence of C1 (blue dots in Fig. 4a) for North Atlantic Deep Water (NADW) is related to the high load of terrestrial fluorescent materials transported by the Arctic5,13,14 rivers, whereas the cause of the high value for the Mediterranean water (MW) is due to the low proportion of this water mass (9 ± 14%) compared to the proportion of NADW (76 ± 33%) in the same sample. Therefore, it is expectable that the archetypal concentration of C1 that our data set produces for the MW should be close to the NADW archetype. For C2, the North Pacific Subtropical Mode Water (STMWP; green dot in Fig. 4b) also departed from the general archetypal C2–AOU trend. Archetypal C1 and C2 can be modelled with archetypal AOU values using power functions (Fig. 4a,b). Given that the high fluorescence of NADW and MW in C1 and of STMWP in C2 is not related to ageing, these water masses were excluded from their corresponding regression models. It is noticeable that the power factor for C1 (0.51 ± 0.04) is almost twice than for C2 (0.31 ± 0.04), indicating a higher C1 production rate per unit of consumed oxygen. Furthermore, in hypoxic waters (dissolved oxygen concentrations <60 µM (ref. 15); orange dots in Fig. 4a,b), C1 and C2 also behave differently; whereas C1 production was enhanced, C2 did not change substantially.

In contrast, we do not observe a significant relationship between the archetypal values of the tryptophan-like fluorescence component (C3) and AOU ($R^2 = 0.001, n = 22, P = 0.88$; Fig. 4c). This lack of correlation is caused by the low archetypal
fluorescence ($<4 \times 10^{-3}$ RU) of the relatively young (AOU $<75$ μmol·kg$^{-1}$) central waters of the Indian and South Pacific oceans (ICW$_{13}$, STMW$_{1}$, SAMW, SPCW$_{20}$ and STMW$_{3p}$; Table 1; purple dots in Fig. 4c). However, such low archetypal values are not observed in the tyrosine-like component (C4), leading to a weak but significant inverse power relationship with AOU (Fig. 4d). The archetypal tyrosine-like fluorescence of the aged Central waters (AOU $>200$ μmol·kg$^{-1}$) of the Equatorial (13EqPac) and Central North Pacific (CMWNP) exceeded the expected value from their AOU (cyan dots in Fig. 4d). These two WT were excluded from the regression model.

**Net FDOM production and turnover times.** On the basis of the relationships observed between the archetypal fluorescence intensity of three out of four fluorescent components (C1, C2 and C4) and AOU, we calculated the net production rate of each component, termed net FDOM production (NFP). A positive value of NFP indicates net production, as for the case of the humic-like components C1 and C2, and a negative value indicates net decay, as for the case of the amino-acid-like C4. The NFP of each component was calculated by multiplying the WT proportion-weighted average fluorescence production values per unit of AOU by the oxygen consumption rate (OCR) for the dark ocean (see Methods). Here we have used a conservative OCR estimate of 0.827 pmol O$_2$ per year$^{19}$. The net humic-like fluorescence production rate obtained was $2.8 \pm 0.2 \times 10^{-5}$ Raman units per year (RU per year) for C1 and 1.5 $\pm$ 0.1 $\times 10^{-6}$ RU per year for C2, whereas C4 was consumed at a net rate of $-1.3 \pm 0.2 \times 10^{-3}$ RU per year in the dark global ocean.

Turnover times of components C1, C2 and C4 were calculated dividing the WT weighted-average fluorescence values of the dark global ocean by its corresponding NFP rate (see Methods). These values represent the time required to produce (C1, C2) or consume (C4) a fluorescence signal of the same intensity as the actual fluorescence of the dark ocean. The resulting turnover of C2, 610 ± 55 years, was significantly longer than the turnover of C1, 435 ± 41 years, and both exceeded the turnover times of the bulk DOC pool—estimated in 370 years$^{11}$—and the terrestrial
DOC in the open ocean—estimated in <100 years$^{17}$—as well as the renewal time of the dark ocean (water depths >200 m)—estimated in 345 years$^9$. Conversely, the turnover time of the tyrosine-like component C4 in 379±103 years was compatible with the turnover time of the bulk DOC.

**Discussion**

The global pattern in the dark ocean of an increase in the humic-like components concomitant to water mass ageing (high AOU values) has been previously reported$^{5,6,18,19}$. Although it has been recently hypothesized that this relationship could also be caused by further transformations of terrestrial humic-like materials in the open ocean$^{20}$, culture experiments have unequivocally demonstrated that these materials can be produced *in situ* in the oceans$^{21}$. In fact, we observe positive and significant relationships between the archetypes of both humic-like components (C1 and C2) and the AOU (Fig. 4a,b). The higher C1 production rate per unit of consumed oxygen in comparison with C2 could be related to different mechanisms of production$^6$ that might be linked to the phylogenetic nature of producers (bacteria, archaea or eukarya)$^{22}$ and/or the sensitivity to environmental oxygen concentration.

The particularly high archetypal fluorescence of C1 for North Atlantic Deep Water (NADW) has been previously described and appears to be clearly related to the high load of FDOM of terrestrial origin transported from the Arctic rivers to the North Atlantic Ocean$^{6,13,14}$ with a relevant proportion of unaltered high molecular weight lignin$^{23}$. However, the cause of the high C2 signature for the North Pacific Subtropical Mode Water has not been previously reported. We hypothesize that it could be related to intense rainfall south of the Kuroshio extension where these water masses are formed$^{24}$, since it is known that rainwater is particularly enriched in these fluorescent compounds$^{25,26}$ and this WT is very shallow (archetypal depth = 277 ± 84 m; Table 1), which means that rainwater would dilute in a few tenths of metres during formation of that warm water mass. Indeed, lignin-derived phenols, highly modified by photo-oxidation, have been found in dissolved and submicron particles suspended in the North Pacific Subtropical Mode Water, suggesting an aerosol source for these fluorescent materials$^{27}$. The high archetypal tyrosine-like fluorescence (C4) of the aged Central waters (AOU > 200 μmol kg$^{-1}$) of the Equatorial (13EqPac) and Central North Pacific (CMW$_{NP}$) might be due also to both WT that occupy shallow layers (archetypal depths 253 ± 13 m for CMW$_{NP}$ and 483 ± 35 m for 13EqPac; Table 1), where protein-like fluorescence is higher because of the proximity to the epipelagic waters, where these materials are usually produced$^6$.

The turnover times of the fluorescent materials (timescale of centuries) are of the same order of magnitude of the turnover time of the bulk DOC$^{11}$, but an order of magnitude faster than the apparent age of the ocean DOC as derived from $^{14}$C measurements (timescale of millennia)$^2$. However, it should be noted that mean age, derived from $^{14}$C involved by nuclear tests, is not homologous with turnover time (mean transit time), derived from total reservoir and fluxes entering/leaving the reservoir.

It is remarkable that the observed decrease in the tyrosine-like fluorescence in the dark ocean is at centennial timescales. It has been reported that the turnover of these fluorophores in the surface ocean is on a timescale of days$^{6,28}$, but this long-term decline in tyrosine-like fluorescence in the dark global ocean, coupled to water mass ageing, has never been reported. We can hypothesize that a minor fraction of the tyrosine-like fluorescence is processed on the scale of centuries, whereas the bulk of the signal has a turnover time on the order of days to weeks. Furthermore, this apparent discrepancy could also be related to the different turnover of the set of compounds that are represented by this fluorescence signature$^{29}$.

We conclude that humic-like fluorescence (C1 and C2) reveals a suitable marker of the production of optically active RDOM with turnover times of 400–600 years. Using the oceans as an incubator, our measurements indicate that the *in situ* microbial production of fluorescent humic-like materials in the dark global ocean, coupled to water mass ageing, has never been reported. We can hypothesize that a minor fraction of the tyrosine-like fluorescence is processed on the scale of centuries, whereas the bulk of the signal has a turnover time on the order of days to weeks. Furthermore, this apparent discrepancy could also be related to the different turnover of the set of compounds that are represented by this fluorescence signature$^{29}$.

We conclude that humic-like fluorescence (C1 and C2) reveals a suitable marker of the production of optically active RDOM with turnover times of 400–600 years. Using the oceans as an incubator, our measurements indicate that the *in situ* microbial production of fluorescent humic-like materials in the dark global ocean, coupled to water mass ageing, has never been reported. We can hypothesize that a minor fraction of the tyrosine-like fluorescence is processed on the scale of centuries, whereas the bulk of the signal has a turnover time on the order of days to weeks. Furthermore, this apparent discrepancy could also be related to the different turnover of the set of compounds that are represented by this fluorescence signature$^{29}$.

We conclude that humic-like fluorescence (C1 and C2) reveals a suitable marker of the production of optically active RDOM with turnover times of 400–600 years. Using the oceans as an incubator, our measurements indicate that the *in situ* microbial production of fluorescent humic-like materials in the dark global ocean, coupled to water mass ageing, has never been reported. We can hypothesize that a minor fraction of the tyrosine-like fluorescence is processed on the scale of centuries, whereas the bulk of the signal has a turnover time on the order of days to weeks. Furthermore, this apparent discrepancy could also be related to the different turnover of the set of compounds that are represented by this fluorescence signature$^{29}$.

We conclude that humic-like fluorescence (C1 and C2) reveals a suitable marker of the production of optically active RDOM with turnover times of 400–600 years. Using the oceans as an incubator, our measurements indicate that the *in situ* microbial production of fluorescent humic-like materials in the dark global ocean, coupled to water mass ageing, has never been reported. We can hypothesize that a minor fraction of the tyrosine-like fluorescence is processed on the scale of centuries, whereas the bulk of the signal has a turnover time on the order of days to weeks. Furthermore, this apparent discrepancy could also be related to the different turnover of the set of compounds that are represented by this fluorescence signature$^{29}$.

We conclude that humic-like fluorescence (C1 and C2) reveals a suitable marker of the production of optically active RDOM with turnover times of 400–600 years. Using the oceans as an incubator, our measurements indicate that the *in situ* microbial production of fluorescent humic-like materials in the dark global ocean, coupled to water mass ageing, has never been reported. We can hypothesize that a minor fraction of the tyrosine-like fluorescence is processed on the scale of centuries, whereas the bulk of the signal has a turnover time on the order of days to weeks. Furthermore, this apparent discrepancy could also be related to the different turnover of the set of compounds that are represented by this fluorescence signature$^{29}$.

We conclude that humic-like fluorescence (C1 and C2) reveals a suitable marker of the production of optically active RDOM with turnover times of 400–600 years. Using the oceans as an incubator, our measurements indicate that the *in situ* microbial production of fluorescent humic-like materials in the dark global ocean, coupled to water mass ageing, has never been reported. We can hypothesize that a minor fraction of the tyrosine-like fluorescence is processed on the scale of centuries, whereas the bulk of the signal has a turnover time on the order of days to weeks. Furthermore, this apparent discrepancy could also be related to the different turnover of the set of compounds that are represented by this fluorescence signature$^{29}$.
conductivity–temperature–depth (CTD) and oxygen sensors installed in the rosette sampler. Salinity and dissolved oxygen were calibrated against bottle samples determined on board with a salinometer and the Winkler method, respectively. The AOU was calculated as the difference between the saturation and measured dissolved oxygen. Oxygen saturation was calculated from salinity and potential temperature. Bottle depths were chosen on the basis of the CTD-O2 profiles to cover as much water masses as possible of the dark global ocean. Seawater samples for fluorescence measurements, collected in 12 l Niskin bottles, were immediately poured into glass bottles and stored in dark conditions until measurement within 6 h from collection. We collected 800 water samples from 200 to 4,000 m depth.

**Figure 3 | Global distribution of the AOU and fluorescence components.** The AOU (a) and the fluorescence intensity at the excitation-emission maxima of each component (Fmax) of the four components (b–e) discriminated by the PARAFAC analysis in the global ocean data set of the Malaspina 2010 Expedition are plotted. Note that the depth range starts at 200 m. See Methods and Supplementary Fig. 2 for a detailed description of the four fluorescence components.
Fluorescence spectral acquisition. When the coloured fraction of marine DOM (CDOM) is irradiated with ultraviolet light, it emits a fluorescence signal characteristic of both amino-acid- and humic-like compounds, which is collectively termed FDOM. Fluorescence excitation–emission matrices (EEMs) were collected with a JY-Horiba Spex Fluoromax-4 spectrofluorometer at room temperature (around 20°C) using 5 nm excitation and emission slit widths, an integration time of 0.25 s, an excitation range of 240–450 nm at 10 nm increments and an emission range of 300–560 nm at 2 nm increments. To correct for lamp spectral properties and to compare results with those reported in other studies, spectra were collected in signal-to-reference (S:R) mode with instrument-specific excitation and emission corrections applied during collection, and EEMs were normalized to the Raman area (RA). In our case, the RA and its baseline correction were performed with the emission scan at 350 nm of the Milli-Q water blanks and the area was calculated following the trapezoidal rule of integration.

To track the variability of the instrument in the Raman, protein- and humic-like regions of the spectrum during the 147 working days of the expedition and assess
gradual spectral bias, three standards were run daily: (1) a P-terphenyl block
(Strana) that fluoresces in the protein region, between 310 and 600 nm exciting at
295 nm; (2) a tetraphenyl butadiene block (Strana) that fluoresces in the humic
region, between 365 and 600 nm exciting at 348 nm; and (3) a sealed Milli-Q
vuvette (Perkin Elmer) scanned between 365 and 450 nm exciting at 350 nm.
Supplementary Figure 1a shows that the temporal evolution of the RA of the Milli-
Q water used on board and the reference P-terphenyl and tetraphenyl
butadiene materials were parallel, which confirms that the Raman normalization
was successful in both the protein- and the humic-like regions of the EEMs.
Therefore, no additional drift corrections were necessary. The comparison between
the reference sealed Milli-Q (sMQ) and the daily Milli-Q water allowed us to
demonstrate that the Milli-Q water was unperturbed using on board and the reference P-terphenyl and tetraphenyl
butadiene materials were parallel, which confirms that the Raman normalization
was successful in both the protein- and the humic-like regions of the EEMs.

Global PARAFAC modelling. PARAFAC was used to identify the fluorescent
components that comprise the EEMs in the global ocean. PARAFAC was per-
formed using the DOMFluor_1.7 Toolbox. Before the analysis, Rayleigh scatter bands
(first order at each wavelength pair where Ex = Em ± bandwidth; second
order at each wavelength pair where Em = 2 × Ex ± (2 × bandwidth)) were
trimmed. The global PARAFAC model was derived based on 1,574 corrected EEMs
and was validated using split-half validation and random initialization. A four-
component model was obtained (Supplementary Fig. 2), two of them of humic-like
nature, peak A/C (at Ex/Em <270–370/470 nm) and peak M (at Ex/Em 320/
400 nm), and two of amino-acid-like nature, peak A/C (at Ex/Em 270/370 nm) and peak M (at Ex/Em 270/370 nm). The tight relationship between
fluorescence (Fmax) in Raman units and Raman units (RU) (refs. 31,32).

Multi-parameter water mass analysis. The dark ocean (from 200 m to the
depth) can be described by the mixing of prescribed WT, characterized by a
unique combination of thermohaline and chemical property values. Water mass
analysis quantifies the proportions of the WT that contribute to a given water
sample. In our case, we have characterized the WT on the basis of its salinity (S)
and potential temperature (θ), which are assumed to be conservative parameters
and, therefore, do not change from the area where the WT are defined to the
study area. The equations to be solved for a water sample j are:

\[ \theta_j = \sum_{i} x_{ij} \theta_i \]  
\[ S_j = \sum_{i} x_{ij} S_i \]  

where \( x_{ij} \) is the proportion of WT i in sample j; \( \theta_i \) and \( S_i \) are the thermohaline
characteristics of sample j and \( \theta_0 \) and \( S_0 \) are the prescribed thermohaline
characteristics of WT in the area where it is defined. Furthermore, the solution of
the multi-parameter water mass analysis includes an additional constraint, that is,
all contributions (\( x_{ij} \)) have to be non-negative.

We identified 18 water masses and 22 WT on the route sampled and their
characteristics are summarized in Supplementary Table 1. They were divided into
three domains according to their depth: central, intermediate and abyssal waters. In
the central domain, there are Eighteen Degrees Water (EDW), Eastern North
Atlantic Central Water (ENACW), defined by two WT of 12 and 15 °C, Equatorial
Atlantic Central Water (13 °C; EEqA8), South Atlantic Central Water (SACW),
defined by two WT of 12 and 18 °C, Indian Subtropical Mode Water (STMW),
Indian Central Water (15 °C; ICW), South Pacific Subtropical Mode Water
(SPMW), South Pacific Central Water (20 °C; SPCW), Equatorial Pacific
Central Water (13 °C; EEqPac), North Pacific Subtropical Mode Water (SPMW)
and North Pacific Central Water (12 °C; CMWP). In the intermediate domain, we
found Mediterranean Water (MW), Sub-Antarctic Mode Water (SAMW), Antarctic
Intermediate Water (AAIW), defined by two WT of 3.1 and 5.0 °C, and North Pacific
Intermediate Water (NPW). In the abyssal domain, there are Circumpolar Deep
Water (1.6°C; CDW), North Atlantic Deep Water (NADW), defined by two types of
2 and 4.6 °C, and Antarctic Bottom Water (AABW).

Equations (1)–(3) can be solved for a maximum of three WT simultaneously.
Given that we have identified 22 WT, we have grouped the WT in the triads
presented in Supplementary Table 2 on the basis of reasonable vertical and
geographical constraints to the water mass mixing usually applied in the analysis of
water masses. Concerning the vertical constraints, for a given region of the ocean,
every WT will mix only with the WT situated immediately above and below
according to their density. Concerning the geographical constraints, every WT will
mix preferentially with WT in their surroundings.

The multi-parameter water mass analysis was applied to the 800 samples from the
dark ocean (θ < 18 °C, AOU > 0) where corresponding measurements of
fluorescence (Fmax1, Fmax2, Fmax3 and Fmax4) and AOU were obtained.

Once the WT proportions (\( x_{ij} \)) are known, the proportion of the total volume of
water sampled that corresponds to WT i (%VOLi) can be calculated as:

\[ \sum_{j} x_{ij} \times x_{ij} \times AOU \]  

Where \( n = 800 \) is the number of deep samples.

Archetypal values of Fmax and AOU for each WT. Using the measured values of
fluorescence and AOU (\( N_i \)) and the proportions of the 22 WT identified in this
study (\( x_{ij} \)), the water mass proportion-weighted average concentration of N in each
WT, \( N_i \), termed archetypal value of \( N_i \), can be calculated as:

\[ \frac{\sum_{j} x_{ij} \times N_i}{\sum_{j} x_{ij}} \]  

where \( N_i \) is the concentration of N in sample j.

The s.d. of the estimated archetypal value of \( N_i \) was obtained by:

\[ \text{s.d.} (N_i) = \sqrt{\frac{\sum_{j} (N_i - \text{archetypal value})^2}{\sum_{j} x_{ij}}} \]  

where \( \text{archetypal value} \) is the proportional contribution of WT i to the
mean fluorescence (Fmax) in Raman units.

NFP and turnover times of the components. The tight relationship between
fluorescence intensity and AOU for components C1, C2 and C4 allows estimating
the rate of change of Fmax per AOU unit (\( \text{OCR} \)). It was calculated as the
first derivative of the power functions fitting Fmax and AOU, (Fig. 4). Then, the
net global production of each fluorescence component, NFP (in RU per year), was
calculated as:

\[ \frac{\sum_{i} \text{VOL}_i \cdot (\text{Fmax} / \text{AOU})}{100 \times \text{OCR}} \]  

where \( \text{VOL}_i \) is the volume of water in the ocean at a depth greater than 200 m.

Once the NFP of the three components was obtained, we calculated their
respective turnover time (\( \tau \)):

\[ \frac{\sum_{i} \text{VOL}_i \cdot \text{Fmax} / \text{AOU}}{100 \times \text{NFP}} \]  

Where \( \text{VOL}_i \cdot \text{Fmax} / \text{AOU} \) is the water volume-weighted average fluorescence of
the dark global ocean.

References


Acknowledgements

We thank C.M. Duarte for the coordination of the Malaspina expedition; the members of the physical oceanography party for collecting, calibrating and processing the CTD data; the chief scientists of the seven legs, the staff of the Marine Technology Unit (CSIC-UTM) and the Captain and crew of R/V Hespérides for their outright support during the circumnavigation; N. Mladenov for the initial protocols; F. Iuculano for fluorescence measurements; and Cintia L. Ramon for support with Matlab software. This study was financed by the Malaspina 2010 circumnavigation expedition (grant number CSD2008–0077). C.R.–C. acknowledges funding through a Beatriu de Pinos postdoctoral fellowship from the Generalitat de Catalunya. M.N.–C. was funded by the CSIC Program ‘Junta para la Ampliación de Estudios’ cofinanced by the ESF.

Author contributions


Additional information

Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing financial interests: The authors declare no competing financial interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/