Bioavailability and bacterial degradation rates of dissolved organic matter in a temperate coastal area during an annual cycle

Christian Lønborg [a,b*], Keith Davidson [a], Xosé A. Álvarez–Salgado[b] and Axel E.J. Miller [a]

[a]Scottish Association for Marine Science, Dunstaffnage Marine Laboratory Oban, Argyll, PA37 1QA, United Kingdom

[b]CSIC, Insituto de Investigacións Mariñas, Eduardo Cabello 6, 36208 Vigo, Spain

[*]Corresponding author:

Tel. +34 986 231 930

Fax. +34 986 292 762

Email: clonborg@gmail.com

Running title:

Bioavailability and bacterial degradation of DOM

Abstract

The bioavailability and bacterial degradation rates of dissolved organic matter (DOM) were determined over a seasonal cycle in Loch Creran (Scotland) by measuring the decrease in dissolved organic carbon (DOC), nitrogen (DON) and phosphorous (DOP) concentrations during long–term laboratory incubations (150 days) at a constant temperature of 14ºC. The experiments showed that bioavailable DOC (BDOC) accounted for 29 ± 11 % of DOC (average ± SD), bioavailable DON (BDON) for 52 ± 11% of DON and bioavailable DOP (BDOP) for 88 ± 8 % of DOP. The seasonal variations in DOM concentrations were mainly due to the bioavailable fraction. BDOP was degraded at a rate of 12 ± 4 % d^{-1} (average ± SD) while the degradation rates of BDOC and BDON were 7 ± 2 % d^{-1} and 9 ± 2 % d^{-1} respectively, indicating a preferential mineralization of DOP relative to DON and of DON relative to DOC. Positive correlations between concentration and degradation rate of DOM suggested that the higher the concentration the faster DOM would be degraded. On average, 77 ± 9 % of BDOP, 62 ± 14 % of BDON and 49 ± 19 % of BDOC were mineralized during the residence time of water in Loch Creran, showing that this coastal area exported C–rich DOM to the adjacent Firth of Lorne. Four additional degradation experiments testing the effect of varying temperature on bioavailability and degradation rates of DOM were also conducted throughout the seasonal cycle (summer, autumn, winter and spring). Apart from the standard incubations at 14ºC, additional studies at 8ºC and 18°C were also conducted. Bioavailability did not change with temperature, but degradation rates were stimulated by increased temperature, with a Q_{10} of 2.6 ± 1.1 for DOC and 2.5 ± 0.7 for DON (average ± SD).

Keywords: DOM, bioavailability, refractory, stoichiometry, mineralization

1. Introduction

Dissolved organic matter (DOM) affects the function of aquatic ecosystems by influencing carbon (Søndergaard and Middelboe, 1995) and nutrient budgets (Bronk, 2002; Karl and Björkman, 2002). In coastal waters, DOM sources include phytoplankton exudation, cell lysis, loss by zooplankton feeding and terrestrial allochthonous matter (Zweifel et al., 1995; Nagata, 2000). Previous work suggests that both autochthonous and allochthonous DOM can be metabolized by bacteria (Tranvik, 1988; Moran and Hodson, 1990), with microbial availability depending on molecular size distribution (Amon and Benner, 1996) and chemical composition (Benner and Opsahl, 2001).

Four processes of importance have been identified to remove DOM from the water column in coastal areas: (1) Photochemical reactions, where DOM is degraded to CO_2 or to compounds bioavailable for bacterial uptake (Moran and Zepp, 1997); (2) Loss via aggregation of DOM due to changes in ionic strength when freshwater mixes with sea water (Sholkovitz, 1976); (3) DOM sorption to particles (Chin et al. 1998); and (4) Bacterial uptake and utilization of the bioavailable fraction (Bronk, 2002; Karl and Björkman, 2002). This article will address the bioavailability and bacterial degradation rates of carbon, nitrogen and phosphorus DOM pools, as few studies have quantified them on seasonal scales.

Earlier studies have revealed that bacterial uptake of bioavailable DOM (BDOM) does not necessarily follow production, leading to accumulations of these materials (Thingstad et al., 1997). This phenomenon is often explained by bacterial nutrient limitation (Williams, 1995; Zweifel et al., 1995), predatory control (Thingstad et al., 1997) and/or a semi–labile nature of BDOM (Søndergaard et al., 2000). Furthermore, temperature could impact on DOM cycling rates by enhancing or depressing bacterial BDOM degradation (Chen and Wangersky, 1996). Consequently, many

processes influence BDOM leading to microbial degradation rates ranging from hours to years (Williams, 2000). The turnover of DOM has been proposed to occur with DOP more bioavailable than DON, which in turn is more bioavailable than DOC (Hopkinson et al., 1997; 2002). This scenario has been used to explain why marine DOM is enriched in C over N and even more depleted in P compared with the Redfield ratio, suggesting that N and P depletion of DOM could be linked with age (Williams 1995). However, recent studies have shown that fresh DOM can have high C:N ratios due to large production of carbohydrates (Fajon et al., 1999), and furthermore some decomposition experiments suggest that DOC could be more degradable than DON (Kragh and Søndergaard, 2004), indicating that the linkage of DOM age with N and P depletion is not valid under all conditions.

Here we present the results of a series of laboratory incubations with the purpose of (1) estimating the bioavailability of DOM (DOC, DON and DOP) over an annual cycle, (2) determining DOM decay rates and (3) assessing the influence of changing temperature on DOM bioavailability and decay in a temperate fjord, Loch Creran (Scotland).

2. Methods

2.1. Study site and water sampling

Water samples were collected on 13 occasions from July 2006 to May 2007 at 5 meters depth with a 10 litre Niskin bottle in the main basin of the fjord Loch Creran, West Scotland (Fig. 1). Loch Creran is 12.8 km long with an average depth of 13 m and a flushing time of 12 days (Tett, 1986). The fjord has a brackish surface layer created by freshwater runoff which is periodically broken down by mixing due to winds or tidal variations (Tett, 1986). Loch Creran is in its dimensions, freshwater input, and tides close to the typical Scottish fjord and is thereby representative for all Scottish lochs (Landless and Edwards, 1976).

2.2. Experimental design

The water was kept in the dark until arrival in the base laboratory, about 1 h after sample collection, where the filtrations and measurements commenced. In the laboratory, water samples were filtered through pre–combusted (450°C for 4 hours) and pre–washed (with both Milli–Q and sampling water) GF/F filters (pore size ~ 0.7 μm) and transferred to 2 litre amber glass bottles. A microbial culture was established by adding an inoculum of GF/C filtered (pore size ~ 1.2 μm) water sample to the GF/F filtrate corresponding to 5 % of the total volume with headspace of ~ 400 ml being left in the incubation flasks. The use of GF/C filters allows the passage of both autotrophic and heterotrophic microbes, but as the incubations were conducted in the dark using amber bottles placed inside darkened boxes, autotrophic organisms were not able to grow in the incubations.

The degradation experiments were conducted in duplicate with a total of six incubations per field sampling: Two bottles received only the inoculum (control bottles), and two bottles received, in addition to the inoculum, carbon ($C_6H_{12}O_6$, 60 μmol L^{-1}) and either nitrate (KNO_3^-, 10 μmol L^{-1}) or phosphate (KH_2PO_4, 1 μmol L^{-1}) to ensure either phosphorus or nitrogen limitation, allowing us to test if enhanced carbon and nutrient levels stimulate DOM bioavailability. The experiments were conducted in the dark, at 14°C for 150 days; this time scale was chosen to include both the labile and parts of the semi-labile DOM pool.

Additional incubation experiments, using the protocol described above, were undertaken at a range of temperatures. These were conducted four times throughout the season (28-Jul, 8-Sep-2006 and 16-Jan, 16-May-2007) with duplicate bottles at each temperature to test if changing temperature would influence the bioavailability and decay rates of DOM. The chosen temperatures were 8 °C to correspond to the average winter temperature; 14°C representing the average summer/autumn temperature, and 18°C which represents a possible future temperature due to global warming. The degradation of DOM was measured as the decrease in DOC, DON and DOP concentrations. During

the incubation period, a minimum of five sub-samples were collected (days 0, 5, 10, 50 and 150) from the duplicated 2 litre amber bottles, using a 50 ml acid washed glass syringe mounted with a 13 mm pre-combusted GF/F filter. The first 10 ml filtrate was discarded whereafter samples were collected to follow the time course concentrations of dissolved inorganic nitrogen (DIN, ammonium + nitrate/nitrite), dissolved inorganic phosphorus (DIP), dissolved organic carbon (DOC), total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP).

All glassware used was first acid–washed in 2M HCl for 24 hours, and then washed 3 times with Milli–Q and incubation water before use. Sub-samples for DOC and TDN analysis were collected directly in pre–combusted (6h) glass ampoules (10 ml) and preserved by adding 10 µl 85 % H_3PO_4. Samples for DIN, DIP and TDP were kept frozen at -20ºC and analyzed within a week after collection.

2.3. Measurements

Samples for chlorophyll *a* and particulate organic matter (POM) determination were collected on precombusted (450°C for 4 hours) 47 mm diameter GF/F filters, dried overnight and thereafter frozen (-20°C). Chlorophyll *a* concentrations were measured in 96 % ethanol after 24 h extraction in the dark, using a Thermo Nicolet Evolution 300 Turn spectrophotometer. Measurements of POC and PON were carried out using a 20-20 ANCA GSL mass spectrometer (PDZ Europa), calibrated with isoleucine. Filters were fumed with concentrated HCl to remove inorganic C and packed in tin disks and injected into the vertical quartz furnace of the analyser and were combusted to CO_2, N_2 and H_2O (Nieuwenhuize et al. 1994).

Particulate phosphate (POP) was determined by suspending the filters in 20 ml of Milli-Q water and wet-oxidizing in acid persulphate for 90 min. Particulate-P was measured as liberated orthophosphate with the standard molybdenium blue technique (Hansen and Koroleff 1999).

DOC/TDN samples were measured in at least triplicates with a Shimadzu TOC analyzer (Pt–catalyst) connected with an Antek–TN measuring unit. Using the deep ocean reference (Sargasso Sea deep water, 2600 m) we obtained a concentration of 45.7 ± 3.0 μmol C L^{-1} (average ± SD) for DOC and 22.0 ± 1.9 μmol N L^{-1} for TDN. The nominal value for DOC provided by the reference laboratory is 44.0 ± 1.5 μmol C L^{-1}, while the TDN value is 21.1 ± 0.8μmol N L^{-1}. Standards for DOC and TDN were made from potassium hydrogen phthalate (C_6H_4(COOK)–COOH) and glycine (NH_4CH_2COOH), with the concentrations of DOC and TDN calculated using a daily calibration curve with 4 points and subtraction of a blank value. DON concentrations were calculated as the difference between TDN and DIN (DON = TDN – DIN) with the standard error (SE) calculated as the sum of the contributions: $SE^2_{DON} = SE^2_{TDN} + SE^2_{NH4} + SE^2_{NO2/NO3}$.

DIN and DIP were measured in triplicate by automated analysis and manual ammonium molybdate methods, respectively (Hansen and Koroleff 1999). TDP was measured in triplicate by the ammonium molybdate method as inorganic phosphorus after a wet oxidation in acid persulphate (Hansen and Koroleff, 1999). The oxidation efficiency was tested with adenosine 5´–triphosphate (ATP) obtaining recoveries between 85 and 96 %. DOP was calculated as the difference between TDP and DIP (DOP = TDP – DIP) with the SE for DOP calculated as: $SE^2_{DOP} = SE^2_{TDP} + SE^2_{DIP}$.

The kinetics of DOM degradation was described by a first–order exponential decay model taking the refractory pool into account:

$$DOM(t) = BDOM \cdot \exp(-k \cdot t) + DOM(150)$$

Where DOM(t) is the amount of DOM remaining at time t, BDOM is the bioavailable pool (μmol L^{-1}), k the degradation rate (day $^{-1}$), t the time (days) and DOM(150) the remaining pool after 150 days of incubation (in μmol L^{-1}). We defined the bioavailable and refractory DOM pools as the

parameters BDOM and DOM(150) found by the exponential model fitted using the Marquardt–Levenberg algorithm.

The Q_{10} coefficient for the effect of increasing temperature on the DOM degradation rate was calculated as:

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{\frac{10}{T_2 - T_1}}$$

k_1 and k_2 are the degradation rates (day^{-1}) at temperatures T_1 and T_2. Three temperatures were tested: 8, 14 and 18ºC.

Regression analyses were performed using the best–fit between the two variables X and Y obtained by regression model II as described in Sokal and Rohlf (1995). In the cases where the intercept was not significantly different from zero, it was set to zero and a new slope calculated. Prior to the regressions, normality was checked. The confidence level was set at 95% with all statistical analyses conducted in Statistica 6.0.

3. Results and discussion

3.1. Hydrography of Loch Creran

Environmental characteristics of the seasonal cycle in Loch Creran are presented in Fig. 2. Fig. 3 shows the seasonal cycles of DOC, DON and DOP concentrations in Loch Creran, which ranged from 80 to 150 µmol L^{-1} for C, 6 to 12 µmol L^{-1} for N, and 0.1 to 0.2 µmol L^{-1} for P. Chlorophyll *a*, particulate organic matter (POM) (Fig. 2a and c) and DOM (Fig. 3) showed opposite seasonal pattern of those of temperature, salinity and inorganic nutrients (DIN and DIP) (Fig. 2 b) Maximum concentrations were recorded in spring, with high levels maintained during summer stratification and minimum values reached during winter mixing, describing the expected seasonal cycle of a temperate coastal system.

3.2. DOM bioavailability

Since our incubations were closed to new production, they forced the bacterial community to use the BDOM produced in situ prior to these experiments. We defined the refractory DOM pool as the concentration in the sample after 150 days of incubation, although reaching the "true" refractory endpoint would probably require longer incubation times. BDOM ranged from 6 to 65 μmol L^{-1} for C, 2 to 7 μmol L^{-1} for N, and 0.05 to 0.18 μmol L^{-1} for P (Fig. 3). It represented 29 ± 11 % of DOC (average ± SD), 52 ± 11 % of DON and 88 ± 8 % of DOP. Overall, the % bioavailability was highest for DOP followed by DON and DOC.

The contributions of BDOM to the bulk DOM pool in Loch Creran are comparable with literature values for marine waters of 19 ± 12 % for DOC within the wide range of 0 – 72 % found by Søndergaard and Middelboe (1995) using incubations periods from 5 to 7 days. It was somewhat higher than the average value for DON of~ 30 %, reported by Bronk (2002) and Lønborg and Søndergaard (2009) in incubations lasting from 5 to 150 days. Values are also in the top end of those previously reported for DOP by Nausch and Nausch (2006; 2007) in incubations of 4 to 7 days duration. However, Hopkinson et al. (2002), when studying the three DOM pools in the middle Atlantic Bight using long term incubations (180 days), found that BDOM represented 30%, 40% and 80% of the bulk DOC, DON and DOP, respectively. These percentages are quite comparable with the values obtained in this work.

The seasonal cycles of BDOM and DOM were parallel for the C, N and P pools (Fig. 3); with significant positive linear relationships found between DOC/BDOC, DON/BDON and DOP/BDOP (Table1). The slope of the linear regressions were not significantly different from 1 (Table 1), indicating that the seasonal variation of DOC, DON and DOP was in fact due to BDOC, BDON and BDOP, respectively, as previously suggested (Williams, 1995; Nausch and Nausch, 2006; 2007). The intercepts of these regressions indicate the residual concentrations of DOC, DON and DOP (i.e.,

1 the portion that is refractory on 150-day timescales). These were 78 ± 4 μmol L^{-1} for C, 3.6 ± 0.7
2 μmol L^{-1} for N and 0.02 ± 0.01 μmol L^{-1} for P (Table 1). The residual DOC calculated in this study
3 is about twice the in situ DOC concentration in the deep sea (35–45 μmol L^{-1}, Hansell and Carlson,
4 1998) which is generally considered resistant to bacterial degradation. By contrast, the residual
5 concentration of DON was only slightly higher than levels found in the deep sea (< 3μmol L^{-1};
6 Bronk, 2002). For the case of DOP, the residual value is around the detection limit of the method, but
7 resembles the levels found in the incubations in other studies (e.g. Karl and Björkman, 2002; Nausch
8 and Nausch, 2006).
9 BDON and BDOP were correlated with inorganic nitrogen and phosphorus (Table 1) and
10 BDOC was positively linearly related with salinity (Table 2). BDOC, BDON and BDOP showed
11 approximately similar patterns as temperature, chlorophyll *a* and particulate organic matter (Table 2).
12 These results strongly indicate that the differences in DOM bioavailability are related to the seasonal
13 variations in plankton biomass and activity. With higher levels in spring and summer when
14 continental runoff decreases, solar irradiation increases and plankton grow at the expense of the
15 inorganic nutrients accumulated in the water column during the autumn and winter.
16 The slopes of the relationships between BDON/DIN and BDOP/DIP provide an estimate of
17 the mineralized N and P derived from the decomposition of DOM (Hopkinson et al., 1997). This
18 indicates that 60 ± 20 % of DIN in Loch Creran originated from BDON and 18 ± 1 % of the net
19 production of inorganic phosphorous was derived from BDOP (Table 1).
20 Changes in DOM bioavailability have previously been explained by nutrient and temperature
21 limitation, varying terrestrial inputs, biological production of refractory DOM, UV-light, changing
22 bacterial community and chemical composition (Benner and Opsahl, 2001; Del Giorgio and Davis,
23 2003; Kawasaki and Benner, 2006). Recent studies have found that bacterial growth and
24 experimentally determined bioavailability can be limited by nutrients (Pinhassi et al. 1999; Lønborg

& Søndergaard 2009). Our treatments with added carbon and inorganic nutrients exhibited no significant effect in terms of the amounts of DOM measured as bioavailable and refractory (data not shown), suggesting that, in Loch Creran, inorganic nutrients were sufficient for a complete mineralization of the accumulated BDOM.

Low temperatures have been shown to both alter rates and availability of organic matter mineralization, as the ability of an organism to sequester substrate declines at low temperature (Nedwell, 1999). However, we found no differences in DOM bioavailability with varying temperature, suggesting that the amounts mineralized over large time scales (150 days) were temperature independent.

If refractory DOM levels are solely related to changes in terrestrial inflow a significant relationship between the DOM pool remaining after 150 days DOM(150) and salinity would be expected. Such a relation was not found, suggesting that other processes were influencing the refractory DOM pool. Microbial communities have been shown to produce refractory products from labile DOM and inorganic nutrients (Kawasaki and Benner, 2006). These transformations did not appear to be of great importance in our incubations, as the addition of labile carbon and inorganic nutrients did not increase the DOM(150) levels significantly.

Photochemical processes can lead to both reduced and enhanced DOM bioavailability, with the impact probably depending on the source and chemical composition of the DOM (Tranvik and Bertilsson, 2001). In this study the incubations were conducted in the dark and only in situ UV light could have influenced the DOM pool, as a result the impact was not possible to assess here.

Bacterial community composition has been reported to vary both seasonally and during incubation experiments (Pinhassi and Hagström, 2000; Massana et al., 2001). These changes could have occurred in our study, but a major influence seems unlikely as previous studies suggest that the measured DOM bioavailability is independent of the added bacterial population (Janse et al., 2000),

with chemical composition having been shown to largely determine DOM bioavailability (Benner and Opsahl, 2001).

In our study, bioavailability appears to be linked to plankton suggesting that the range of decay rates could be a consequence of differing reactivity of the compounds produced by the plankton community. This could be through plankton seasonal changes in species composition, a coupling between varying community and DOM chemical composition having been suggested by Sun et al. (2004).

3.3. DOM stoichiometry

Fig. 4 shows the significant linear relationships between DOC, DON and DOP ($p < 0.02$). These relations suggest that the time course of the three DOM pools is parallel through the seasonal cycle. The significant origin intercepts of these regressions also suggest that a background level of DOC of 22 ± 10 and 57 ± 8 μmol L^{-1} would persist when DON and DOP reached zero, respectively. Furthermore, the origin intercept of the relationship between DON and DOP shows that 3.4 ± 0.9 μmol L^{-1} of DON would remain when DOP is completely depleted.

The slopes of the regressions provide the average C:N:P molar ratios of DOM: 482 (± 75): 46 (± 17): 1. This can be compared with the average stoichiometry of BDOM, obtained also from the slopes of the significant ($p < 0.05$) linear relationships between BDOC, BDON and BDOP (Fig. 4): 328 (± 54): 42 (± 9): 1. Note that the origin intercept of the regressions between the three BDOM pools is not significantly different from zero, as one might expect considering that approximate background concentrations of 78 ± 4 μmol L^{-1} of DOC, 3.6 ± 0.7 μmol L^{-1} of DON and 0.02 ± 0.01 μmol L^{-1} of DOP are reached after 150 days of incubation (see origin intercepts in Table 1). The overlapping standard errors of the C:N:P molar ratios of DOM and BDOM indicate that they were not significantly different, supporting the suggestion that the slopes of the linear regressions between

DOC, DON and DOP can be used as a proxy of BDOM stoichiometry (e.g. Hopkinson et al., 2002; Álvarez–Salgado et al., 2006).

The C:N molar ratio of BDOM, 8 (± 2), was not significantly different from the Redfield ratio characteristic of the products of synthesis and early degradation of marine phytoplankton (6.7: 1, Redfield et al. 1963), or from phytoplankton produced DOM (11: 1, Conan et al. 2007) as observed in other coastal ecosystems (Hopkinson et al., 1997; 2002; Álvarez–Salgado et al., 2006), strongly indicating the planktonic origin of BDOM. In contrast, the average C:P molar ratio of BDOM, 328 (± 54) was strongly P depleted compared with the Redfield ratio of 106 as observed by other authors (Hopkinson et al., 1997; 2002; Álvarez–Salgado et al., 2006) but within the wide range found for recently produced algae DOM, from ~ 17 to 500 (Conan et al., 2007). When compared with the Redfied ratio of 16, and the ratio of 6.5 for fresh plankton DOM (Conan et al., 2007), the average N:P molar ratio for BDOM, 42 (± 9) again suggests P depletion. High C:N:P ratios of BDOM may either be due to plankton release of C–rich labile compounds such as mono and polysaccharides under in situ N– and/or P–depleted conditions (Williams, 1995; Fajon et al., 1999) or/and in-situ preferential degradation of the N– and/or P–rich compounds (Hopkinson et al., 1997; 2002). The in-situ nutrient levels in Loch Creran (Fig. 2b) as well as the nutrient enrichment experiments carried out in this work suggest that the release by phytoplankton of C–rich labile compounds is not responsible for the observed stoichiometry of BDOM. Furthermore, the significant origin intercepts of the regressions between DOC/DON, DOC/DOP and DON/DOP as well as the high slopes of the regressions between BDOC/BDOP and BDON/BDOP (Fig. 4) show that DOP was mineralised faster than DON and DON faster than DOC in the field, prior to the incubation experiments, which links the C:N:P stoichiometry of BDOM in this area with age (Williams, 1995).

3.4. DOM degradation rates

Table 3 summarises the first order decay constants of the BDOM confined in the incubation bottles which were obtained by fitting the observed exponential degradation of DOC, DON and DOP with time. It should be noted that using this method, the lability of BDOM is a function of the time steps being employed to measure reactivity. Given that our time step is > 5 days and that the number of observation points was 5, the model just considered a singular labile DOM pool. The degradation rate of DOC (k_{DOC}) at 14°C was 6 ± 3 % day $^{-1}$ (average ± SE), ranging from 2 to 12 % day $^{-1}$. k_{DOC} values were maximum in spring/summer and minimum in winter. A significant ($p < 0.006$) positive correlation was found between the decay constants and the initial concentration of BDOM (Table 1), implying that higher BDOM concentrations would lead to faster mineralization rates as observed by Hopkinson et al. (1997) in Georges Bank.

Consistent with other studies (e.g. Garber, 1984), the degradation rates of DOC, DON and DOP followed the same pattern (Table 1). A significant ($p < 0.002$) positive correlation was observed between k_{DOC}, k_{DON} and k_{DOP}, and the corresponding linear regression slopes indicate that DOC and DON were degraded at a rate equivalent to 58 ± 17 % and 78 ± 18 % (slope ± SE) of DOP (Fig. 5). This is in agreement with the general acceptance that the degradation rate of the DOM pools follows the sequence DOP > DON > DOC (Garber, 1984; Hopkinson et al., 1997; 2002). This result is consistent with the previous discussion of C:N:P stoichiometry of the initial BDOM in the incubation flasks, suggesting that fractionation during DOM mineralization occurs before and during the incubation period.

The half–live times of DOC, 7 ± 5 days (average ± SD), DON, 5 ± 3 days and DOP, 3 ± 1 days, calculated from the decay coefficients as the time when half of BDOM was degraded, suggest that DOC decay rates obtained in this study resemble the degradation of semi–labile DOC, while DON and DOP decay rates are closer to those for the degradation of labile DOM found in other studies (Gardner, 1984; Hopkinson et al., 1997; 2002; Kragh and Søndergaard, 2004).

The efficiency of Loch Creran to process the BDOM can be calculated from the exponential decay model using the degradation rates at a typical summer water temperature of 14°C and using the average flushing time of water of 12 days (Tett 1986). We found that 73 ± 9 % of BDOP (average ± SD) is mineralized within the fjord, while only 49 ± 19 % and 62 ± 14 % of DOC and DON are degraded, respectively. The stoichiometry of BDOM degraded within Loch Creran exhibits an average C:N:P molar ratio of 236: 38: 1, with the exported BDOM being more C and N rich, with a C:N:P molar ratio of 581: 59: 1. These estimates suggest that C–rich BDOM would still persist after the water is exported from Loch Creran, and thus could support heterotrophic production in the adjacent Firth of Lorne. This emphasises the importance of the flushing time of water as compared with half–live times of BDOM in determining if DOM is used or exported away from the area where it is produced (Hopkinson et al., 2002).

The decay rates of DOM in Loch Creran could have been further influenced by temperature, as previous studies show inhibition of bacterial growth at low temperatures, probably due to low extra–cellular enzymatic hydrolysis rates (Kirchman and Rich, 1997). The impact of temperature on DOM degradation was measured in this study, showing that increased temperature led to higher decay rates of DOC and DON, while no clear effect was detected for DOP (Table 3; Fig. 6). The Q_{10} values of 2.6 ± 1.1 for DOC (average ± SD) and 2.5 ± 0.7 for DON had largest impact in July, which is consistent with previous studies (Garber, 1984; Chen and Wangersky, 1996). Results from bottle experiments such as these are difficult to extrapolate to in–situ processes, but temperature effects could have important implications due to the relative importance of accumulation (Williams, 1995) versus offshore horizontal transport (Álvarez–Salgado et al., 2006) in coastal areas. From the results obtained here we suggest that low temperatures could be rate limiting for degradation of DOM, implying that DOM will decompose closer or further away from the source depending on the temperature.

4. Conclusions

The main conclusions from this study are: (1) the bioavailable fraction of DOM in the temperate coastal ecosystem of Loch Creran controlled the variability of the bulk DOM, showing a marked seasonal cycle associated to the plankton biomass and activity; (2) the stoichiometry of BDOM suggests fractionation during in situ mineralization of this material prior to incubation, with DOP cycling faster than DON and the latter faster than DOC; (3) the estimated degradation rates of BDOM also indicate that mineralization occurred in the sequence DOP > DON > DOP during the incubation experiments; (4) since the turnover times of BDOM are of the same order of magnitude as the flushing time of water in the fjord, a offshore export of C–rich BDOM is likely; and (5) the chemical composition of BDOM and temperature appear to be the key factors influencing the observed decay rates, with no evidence of an effect of nutrient limitation.

Acknowledgement

This study was funded by a fellowship to CL from the Early Training site ECOSUMMER (Marie Curie action 20501). KD was in receipt of funds from the NERC Oceans 2025 programme. The crew of Seol Mara is thanked for help with the collection of water samples used in this study. Colin Stedmon is acknowledged for comments on the paper. The authors are grateful to the anonymous reviewers and the associate editor who provided valuable comments on the manuscript.

References

Álvarez–Salgado, X.A., Nieto–Cid, M., Gago, J., Brea, S., Castro, C.G., Doval, M.D., Pérez, F.F. 2006. Stoichiometry of the degradation of dissolved and particulate biogenic organic matter in the NW Iberian upwelling. J. Geophys. Res. 111, C07017, doi:10. 1029/2004JC002473.

Amon, R.M.W., Benner, R. 1996. Bacterial utilization of different size classes of dissolved organic matter. Limnology and Oceanography 41, 41-51.

Benner, R., Opsahl, S. 2001. Molecular indicators of the sources and transformations of dissolved organic matter in the Mississippi river plume. Org. Geochem. 32, 597–611.

Bronk, D.A. 2002. Dynamics of dissolved organic nitrogen. In: Hansell, D.A., Carlson C.A. (Eds.), Biogeochemistry of marine dissolved organic matter. Academic Press, USA, pp. 153–247.

Chen, W., Wangersky, P.J. 1996. Rates of microbial degradation of dissolved organic carbon from phytoplankton cultures. J. Plank. Res. 18, 1521–1533.

Chin, W.C., Orellana, M.V., Verdugo, P. 1998. Spontaneous assembly of marine dissolved organic matter into polymer gels. Nature 391, 568-572.

Conan, P., Søndergaard, M., Kragh, T., Thingstad, F., Pujo–Pay, M., Williams, P.J. Le B., Markager, S., Cauwet, G., Borch, N.H., Evans, D., Riemann, B. 2007. Partitioning of organic production in marine plankton communities: The effects of inorganic nutrient ratios and community composition on new dissolved organic matter. Limnol. Oceanogr. 52, 753–765.

Del Giorgio, P., Davies, J. 2003. Patterns of dissolved organic matter lability and consumption across aquatic ecosystems. In: Findlay, S.E.G., Sinsabaugh R.L. (Eds.), Aquatic Ecosystems: Interactivity of Dissolved Organic Matter. Academic Press, USA, pp. 399–424.

Fajon, C., Cauwet, G., Lebaron, P., Terzic, S., Ahel, M., Malej, A., Mozetic, P., Turk, V. 1999. The accumulation of polysaccharides in plankton cells, its excretion and bacterial response during controlled experiments. FEMS Microbiol. Ecol. 29, 351–363.

Garber, J.H. 1984. Laboratory study of nitrogen and phosphorus remineralization during the decomposition of coastal plankton and seston. Est. Coast Shelf Sci. 18, 685–702.

Hansen, H.P., Koroleff, F. 1999. Automated chemical analysis. In: Grasshoff, K., Kermling, K., Ehrhardt M. (Eds.), Methods of seawater analysis. Wiley-VCH, Germany, pp. 159–226.

Hansell, D.A., Carlson, C.A. 1998. Deep–ocean gradients in the concentration of dissolved organic carbon. Nature 395, 263–266.

Hopkinson, C.S., Fry, B., Nolin, A.L. 1997. Stoichiometry of dissolved organic matter dynamics on the continental shelf of the northeastern U.S.A. Cont. Shelf. Res. 17**,** 473–489.

Hopkinson, C.S., Vallino, J.J., Nolin, A.L. 2002. Decomposition of dissolved organic matter from the continental margin. Deep–Sea Res. Part II 49, 4461–4478.

Janse, I., Zwart, G., Van Der Maarel, M.J.E.C., Gottschall, J.C. 2000. Composition of the bacterial community degrading *Phaeocystis* mucopolysaccharides in enrichment cultures. Aquat. Microb. Ecol. 22, 119–133.

Karl, D.M., Björkmann, K.M. 2002. Dynamics of DOP. In: Hansell, D.A., Carlson C.A. (Eds.), Biogeochemistry of marine dissolved organic matter. Academic Press, USA, pp. 249–366.

Kawasaki, N., Benner, R. 2006. Bacterial release of dissolved organic matter during cell growth and decline: Molecular origin and composition. Limnol. Oceanogr. 51, 2170–2180.

Kirchmann, D.L., Rich, J.H. 1997. Regulation of bacterial growth rates by dissolved organic carbon and temperature in the equatorial Pacific Ocean. Microb. Ecol. 33**,** 11–20.

Kragh, T., Søndergaard, M. 2004. The bioavailability of autochthonous DOC: Effects of mesozooplankton. Aquat. Microb. Ecol. 36, 61–72.

Landless, P.J., Edwards, A. 1976. Economical ways of assessing hydrography for fish farms. Aquaculture 8, 29–43.

Lønborg, C., Søndergaard, M. 2009. Microbial availability and degradation of dissolved organic carbon and nitrogen in two coastal areas. Est. Coast Shelf Sci. 81, 513-520.

Massana, R., Pedrós–Alió, C., Casamayor, E.O., Gasol, J.M. 2001. Changes in marine bacterioplankton phylogenetic composition during incubations designed to measure biogeochemically significant parameters. Limnol. Oceanogr. 46, 1181–1188.

Moran, M.A., Hodson, R.E. 1990. Bacterial production on humic and nonhumic components of dissolved organic carbon. Limnol. Oceanogr. 35, 1744–1756.

Moran, M.A., Zepp, R.G. 1997. Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. Limnol. Oceanogr. 42, 1307–1316.

Nagata, T. 2000. Production mechanisms of dissolved organic carbon. In: Kirchman, D.L. (Ed.), Microbial ecology of the oceans. Wiley–Liss, USA, pp. 121–153.

Nausch, M., Nausch, G. 2006. Bioavailability of dissolved organic phosphorus in the Baltic Sea. Mar. Ecol. Prog. Ser. 321, 9–17.

Nausch, M., Nausch, G. 2007. Bioavailable dissolved organic phosphorus and phosphorus use by heterotrophic bacteria. Aquat. Biol. 1, 151 – 160.

Nedwell D.B. 1999. Effect of low temperature on microbial growth: lowered affinity for substrates limits growth at low temperature FEMS Micro. Ecol 30, 101- 111.

Nieuwenhuize, J., Maas Y.E.M., Middelburg J.J. 1994. Rapid analysis of organic carbon and nitrogen in particulate materials. Mar. Chem. 45 :217 -224.

Pinhassi, J., Gomez-Consarnau, L., Alonso-Saez, L., Sala, M.M., Vidal, M., Pedros-Alio, C., Gasol, J.M. 2006. Seasonal changes in bacterioplankton nutrient limitation and their effects on bacterial community composition in the NW Mediterranean Sea. Aquatic Microbial Ecology 44, 241–252.

Pinhassi, J., Hagström, Å. 2000. Seasonal succession in marine bacterioplankton. Aquat. Microb. Ecol. 21, 245–256.

Redfield, A.C., Ketchum, B. K., Richards F.A. 1963. The influence of organisms on the composition of sea–water, In: Hill M. N. (Ed.), The Sea, vol. 2, The Composition of Sea Water: Comparative and Descriptive Oceanography. Wiley–Interscience, USA, pp. 26– 77.

Sokal, F.F., Rohlf, F.J. 1995. Biometry. Freeman, New York.

Sholkovitz, E.R. 1976. Flocculation of dissolved organic and inorganic matter during the mixing of river water and seawater. Geochim. Cosmochim. Acta. 40, 831-845.

Søndergaard, M., Middelboe, M. 1995. A cross–system analysis of labile dissolved organic carbon (DOC–L). Mar. Ecol. Prog. Ser. 118, 283–294.

Søndergaard, M., Williams, P. J. Le B., Cauwet, G., Riemann, B., Robinson, C., Terzoc, S., Woodward, E. M. S., Worm, J. 2000. Net accumulation and flux of dissolved organic carbon and dissolved organic nitrogen in marine plankton communities. Limnol. Oceanogr. 45, 1097–1111.

Sun, M–Y., Zou, L., Dai, J., Ding, H., Culp, R.A., Scranton, M.I. 2004. Molecular carbon isotopic fractionation of algal lipids during decomposition in natural oxic and anoxic seawaters. Org. Geochem. 35, 895–909.

Tett, P. 1986. Physical exchange and the dynamics of phytoplankton in Scottish sea–lochs. In: S. Skreslet (Ed.), The role of Freshwater outflow in coastal marine ecosystems. Springer–Verlag, Germany, pp. 205–218.

Thingstad, T.F., Hagström, Å., Rassoulzadegan, F. 1997. Accumulation of degradable DOC in surface waters: is it caused by a malfunctioning microbial loop?. Limnol. Oceanogr. 42, 398–404.

Tranvik, L.J. 1988. Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of differing humic content. Microb Ecol 16, 311–322.

Tranvik, L.J., Bertilsson, S. 2001. Contrasting effects of solar UV radiation on dissolved organic sources for bacterial growth. Ecol. Lett. 4, 458–463.

Zweifel, U.L., Wikner, J., Hagström, Å., Lundberg, E., Norrman, B. 1995. Dynamics of dissolved organic carbon in a coastal ecosystem. Limnol. Oceanogr. 40, 299–305.

Williams, P. J. Le B. 1995. Evidence for the seasonal accumulation of carbon–rich dissolved organic material, its scale in comparison with changes in particulate material and the consequential effect on net C/N assimilation ratios. Mar. Chem. 51, 17–29.

Williams, P. J. Le B. 2000. Heterotrophic bacteria and the dynamics of dissolved organic material. In: Kirchman, D.L. (Ed.), Microbial ecology of the oceans. Wiley–Liss, USA, pp. 153–200.

Figure Legends

Fig. 1. Map of Scotland with the sampling station (X) in Loch Creran.

Fig. 2. Field conditions in Loch Creran during the experiments during 2006 to 2007, with (a) salinity, temperature, chlorophyll a, (b) dissolved inorganic phosphorus (PO_4^{2-}), ammonium (NH_4^+), nitrate/nitrite (NO_3^-/NO_2^-), (c) particulate organic carbon (POC), nitrogen (PON) and phosphorous (POP) at time of sample collection. Values for nutrients and POM are mean values of 3 replicates ± standard error.

Fig. 3. Temporal variability in total, bioavailable and refractory (a) DOC, (b) DON and (c) DOP. Error bars represent standard errors.

Fig. 4. X–Y plots of (a) DON with DOC (top), BDON versus BDOC (bottom), (b) DOP with DOC (top), BDOP versus BDOC (bottom) and (c) DOP versus DON (top), BDOP versus BDON (bottom). Solid and dashed lines represent the corresponding regression and error bars are standard errors. R^2 = coefficient of determination, p = significant level.

Fig. 5. Plots of the relationship between decay rates (d^{-1}) for BDOM predicted from the exponential decay model with (a) k_{DON} vs. k_{DOC}, (b) k_{DOP} vs. k_{DOC} and (c) k_{DOP} vs. k_{DON}. The dashed line represents a 1:1 line where the decay rates would be equal and the solid lines represent the regression lines found. Error bars represent standard errors.

Fig. 6. The decay at 8 and 18°C of (a) BDOC, (b) BDON and (c) BDOP for sample water collected on the 28 of July 2006 in Loch Creran. The solid line represents the line predicted by the exponential decay model and error bars are standard errors.

Figure(s)

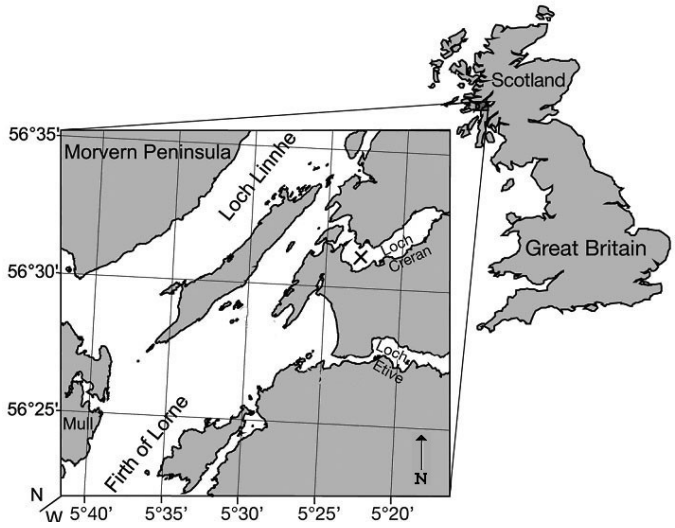


Fig. 1.

Figure(s)

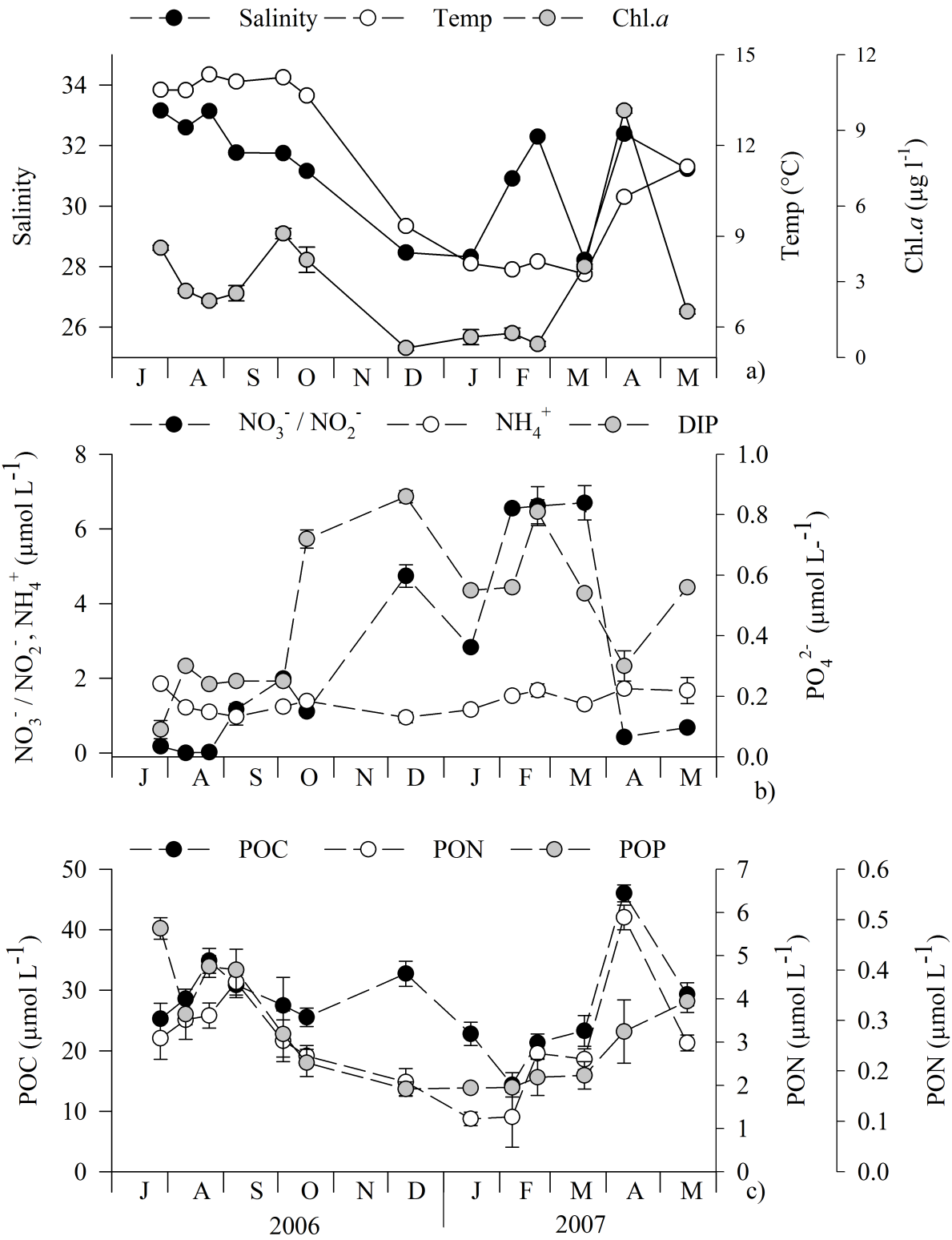


Fig.2.

Figure(s)

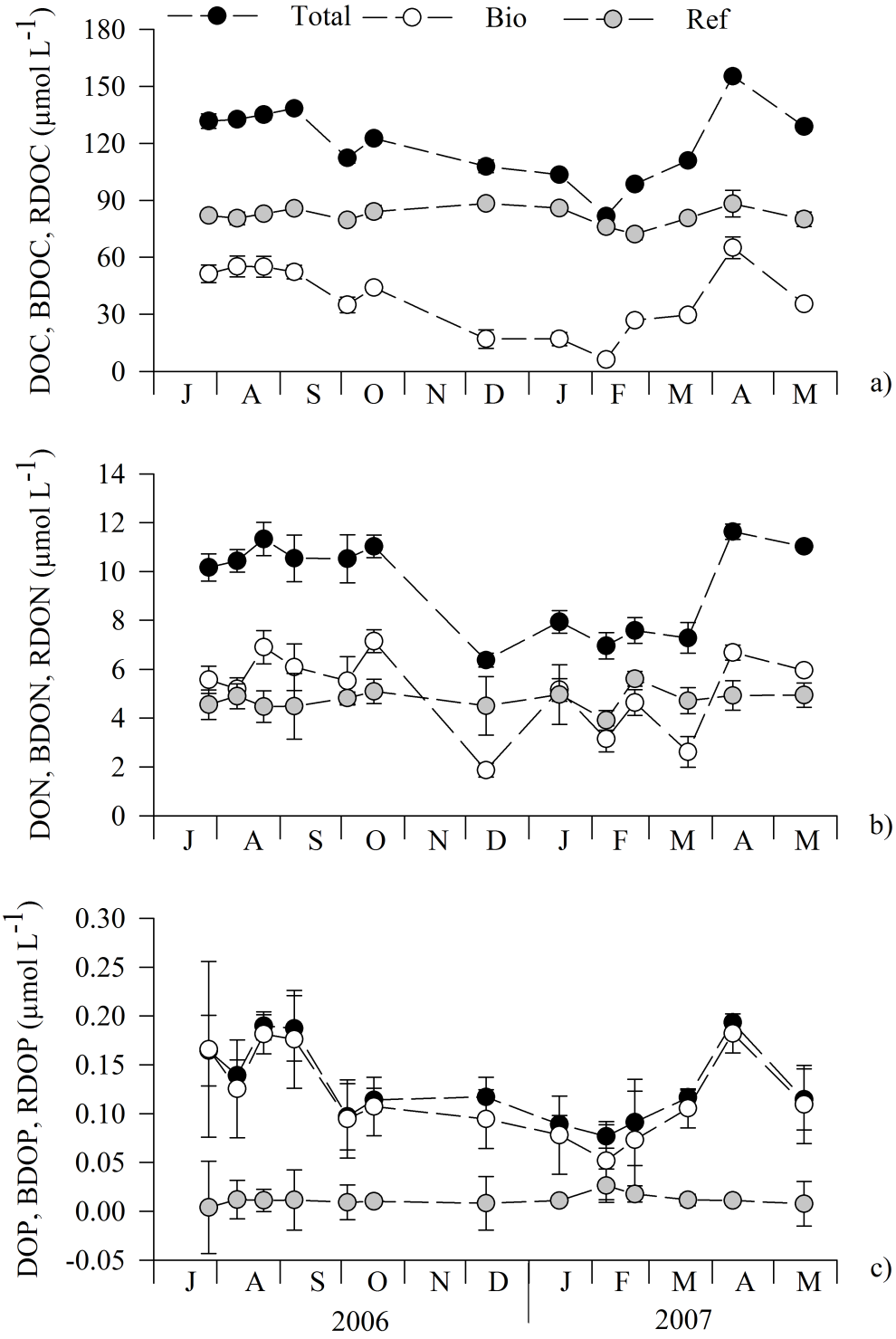


Fig.3.

Figure(s)

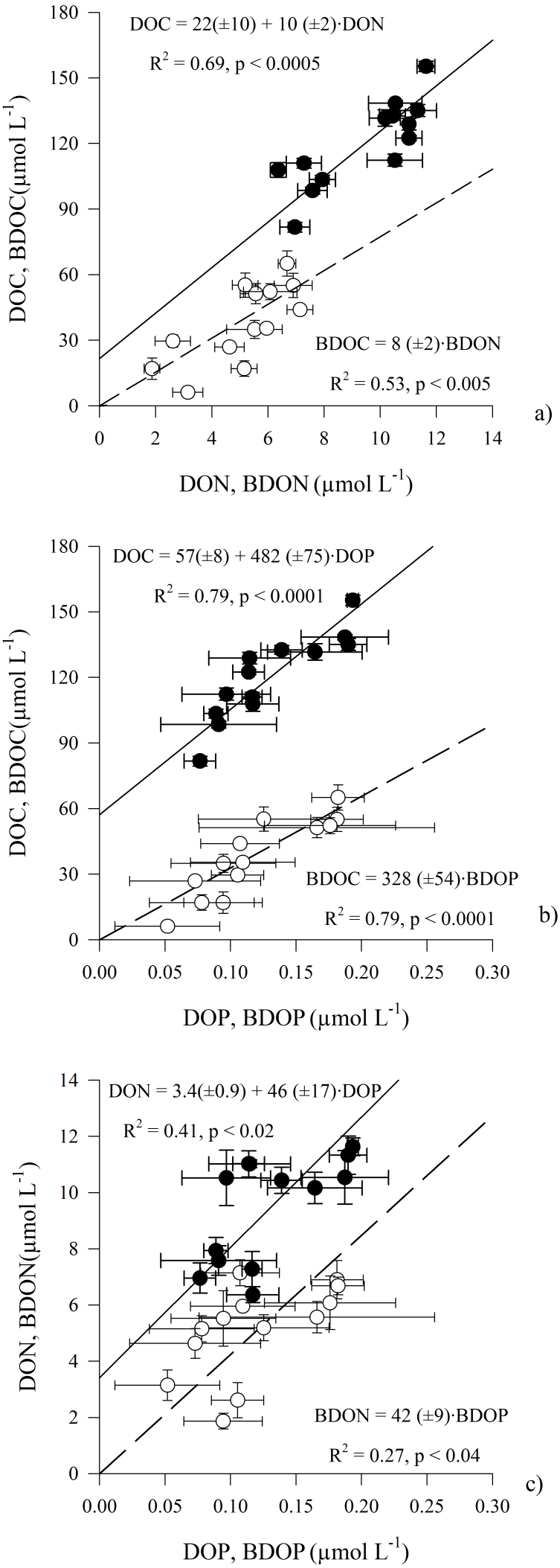
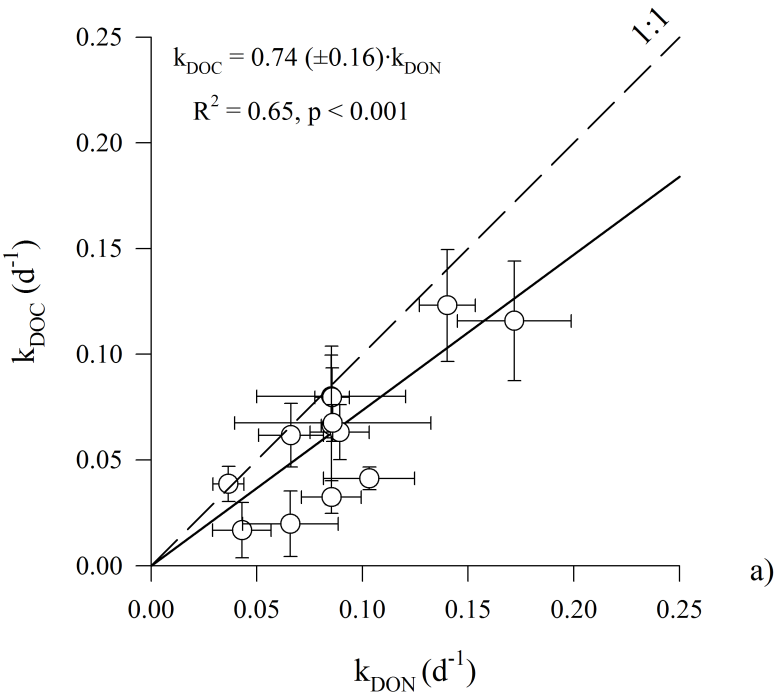
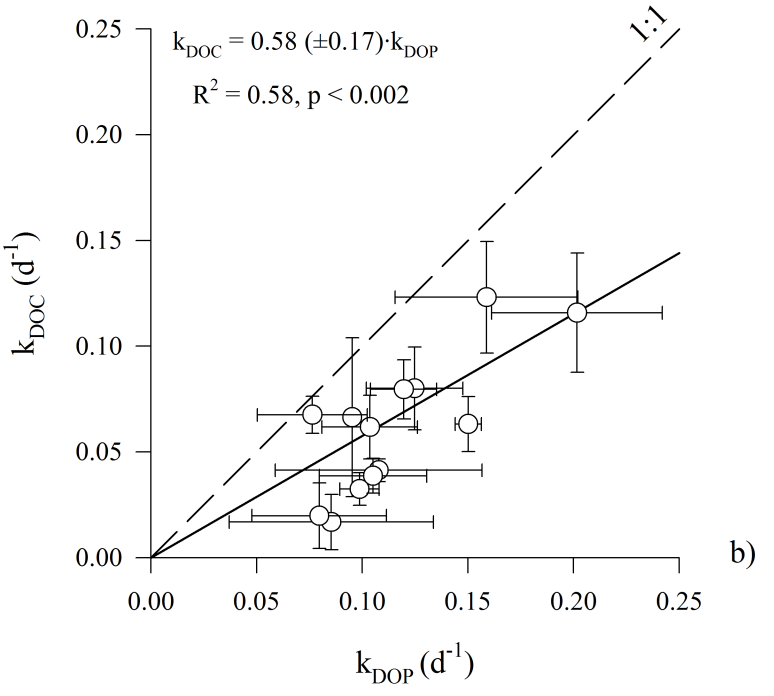


Fig.4.

Figure(s)





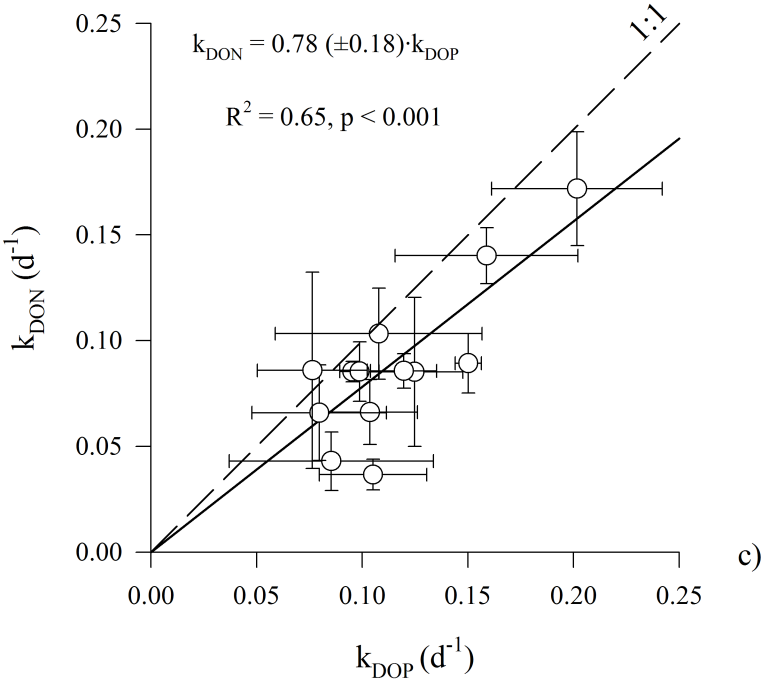


Fig.5.

Figure(s)

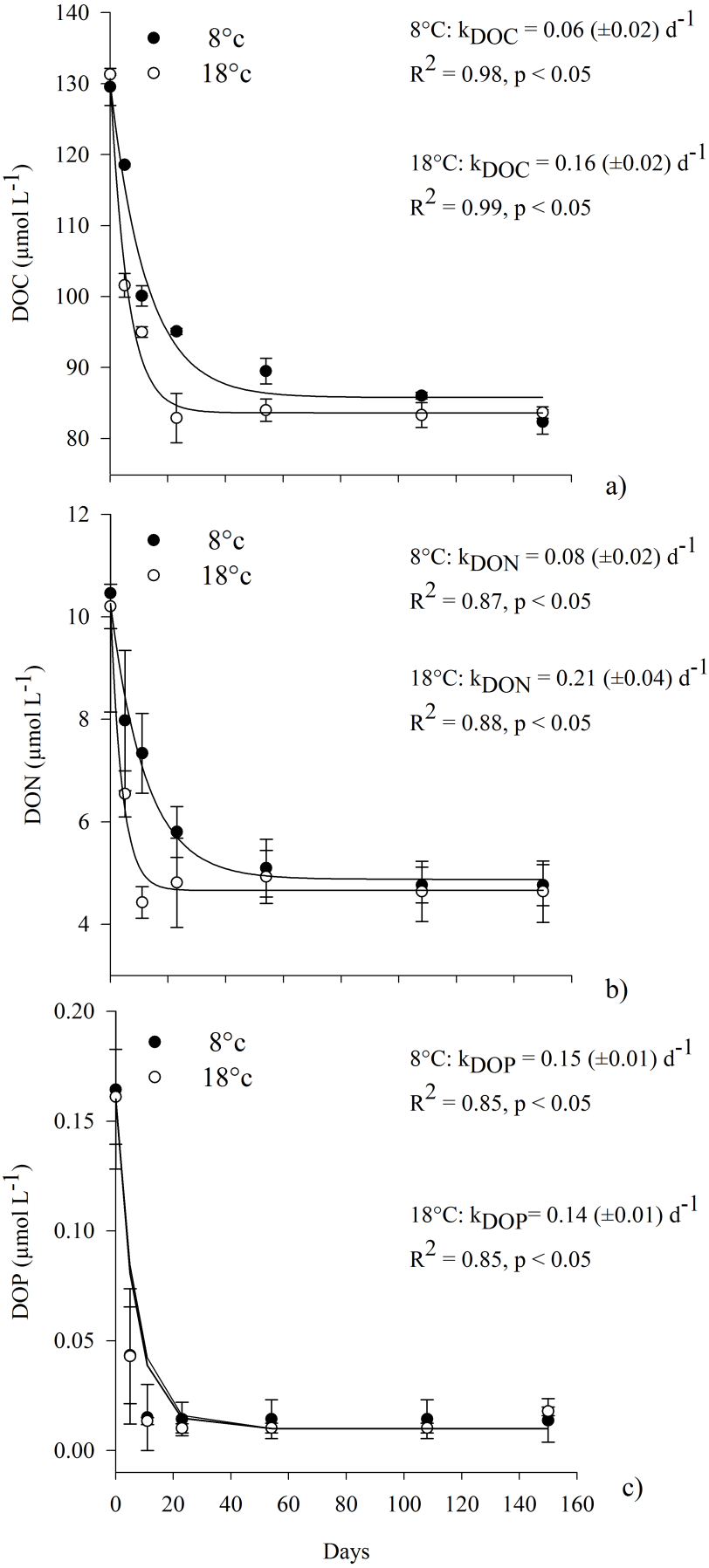


Fig.6.

Table(s)

Table 1

Significant regressions between BDOM and DOM, Inorganic nutrients (DIN, DIP) and degradation constants at 14ºC (k–DOC, k–DON and k–DOP) obtained by fitting the exponential degradation of DOC, DON and DOP with time. Slope, incept, and standard error are values found by Model II regression. R^2 = coefficient of determination, p = significant levels and n.s. – not significant.

X	Y	Slope	Error	Intercept	Error	R^2	p
BDOC	DOC	1.1	±0.1	78	±4	0.89	<0.0001
BDON	DON	1.2	±0.2	3.6	±0.7	0.84	<0.0001
BDOP	DOP	0.93	±0.05	0.019	±0.005	0.97	<0.0001
DIN	BDON	-0.6	±0.2	7.5	±0.6	0.61	<0.002
DIP	BDOP	-0.18	±0.01	0.20	±0.02	0.49	<0.008
BDOC	k-DOC	0.0016	±0.0004	n.s.	n.s.	0.50	<0.006
BDON	k-DON	0.017	±0.005	n.s.	n.s.	0.38	<0.02
BDOP	k-DOP	0.9	±0.1	n.s.	n.s.	0.54	<0.0001

Table 2

Matrix of the correlation coefficient (R^2) of the significant ($p < 0.05$) linear regressions between DOM bioavailability and hydrological data from Loch Creran. n.s. – not significant.

X	Salinity	Temperature	Chl *a*	POC	PON	POP
BDOC	0.39	0.35	0.41	0.46	0.77	0.50
BDON	n.s.	0.30	n.s.	n.s.	0.26	0.27
BDOP	n.s.	0.39	0.27	0.54	0.66	0.60

Table 3

Degradation constants obtained by fitting the exponential degradation of DOC, DON and DOP with time at varying temperature (T, °C) for DOC, DON and DOP ± standard error; R^2 = coefficient of determination; Numbers of points used for the estimate was 5 or 6 in all cases.

Date	T (ºC)	k_{DOC} (% day^{-1})	R^2	k_{DON} (% day^{-1})	R^2	k_{DOP} (% day^{-1})	R^2
28-Jul-2006	8	6 ± 2	0.98	8 ± 2	0.87	15 ± 1	0.85
	14	12 ± 3	0.94	14 ± 1	0.93	16 ± 4	0.82
	18	16 ± 2	0.99	21 ± 4	0.88	14 ± 1	0.85
11-Aug-2006	14	8 ± 2	0.94	9 ± 4	0.83	12 ± 2	0.84
24-Aug-2006	14	12 ± 3	0.97	17 ± 3	0.84	20 ± 4	0.81
8-Sep-2006	8	6 ± 1	0.98	6 ± 1	0.85	7 ± 4	0.76
	14	8 ± 1	0.99	9 ± 1	0.94	12 ± 2	0.81
	18	11 ± 2	0.97	14 ± 2	0.83	16 ± 3	0.77
4-Oct-2006	14	4 ± 1	0.91	10 ± 2	0.85	11 ± 5	0.53
17-Oct-2006	14	6 ± 2	0.87	7 ± 2	0.84	10 ± 2	0.81
11-Dec-2006	14	2 ± 1	0.97	4 ± 1	0.93	9 ± 5	0.82
16-Jan-2007	8	3 ± 1	0.95	4 ± 1	0.87	7 ± 4	0.72
	14	7 ± 4	0.97	9 ± 0	0.96	10 ± 0	0.81
	18	9 ± 5	0.97	11 ± 0	0.86	15 ± 4	0.83
8-Feb-2007	14	2 ± 1	0.75	7 ± 2	0.83	8 ± 3	0.64
22-Feb-2007	14	3 ± 1	0.96	9 ± 1	0.83	10 ± 1	0.66
20-Mar-2007	14	4 ± 1	0.96	4 ± 1	0.87	11 ± 3	0.86
11-Apr-2007	14	6 ± 3	0.92	9 ± 1	0.90	15 ± 1	0.96
16-May-2007	8	5 ± 1	0.92	6 ± 1	0.85	7 ± 5	0.67
	14	7 ± 3	0.91	9 ± 3	0.90	10 ± 3	0.78
	18	9 ± 2	0.92	12 ± 3	0.92	10 ± 6	0.53