# The role of allochthonous inputs of dissolved organic carbon on the hypolimnetic oxygen content of reservoirs

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#### ABSTRACT

Hypolimnetic oxygen content in lentic ecosystems has traditionally been modeled as a function of variables measured at the epilimnion, or that are supposed to drive epilimnetic processes, like total phosphorus load. However, in man-made reservoirs the river inflow can plunge into deep layers, directly linking the hypolimnion with the surrounding watershed. In these circumstances, organic matter carried by the river can influence the hypolimnetic oxygen content without important intervention of epilimnetic processes. Taking long-term data from two reservoirs in Spain, we applied an empirical regression approach to show that the dissolved organic matter carried by the river is the main driver shaping the hypolimnetic oxygen content. By contrast, typical variables commonly included in the modeling of the oxygen content in the hypolimnion (nutrient concentrations, chlorophyll a, and dissolved organic carbon measured in the water column) did not show any significant correlation. Interpretations from this regression approach were supported by a comparison between the monthly oxygen consumption in the hypolimnion and the monthly dissolved organic carbon load from the river inflow. We also revisited the prediction of the year-to-year variability of the Nürnberg's anoxic factor in four reservoirs from Spain and USA, explicitly including the allochthonous sources in the equations. These sources were significant predictors of the anoxic factor, especially in those systems subject to relatively high human impact. Thus, effects of allochthonous dissolved organic carbon should always be considered in empirical modeling and management of reservoir hypolimnetic processes related to oxygen content (e.g. anoxia, nutrient internal loading, or phosphorus cycle resilience).

**Key words:** labile organic carbon; C:N ratio; chloride; anoxic factor; empirical modeling; DOC; reservoir; hypolimnion; oxygen

#### **INTRODUCTION**

Ecologists have long recognized that lacustrine ecosystems are open systems, especially after the seminal work by Vollenweider (1968), in which the principal role of the external nutrient loading in the eutrophication of surface waters was first systematically explored. Later, the prevalence of phosphorus as the limiting factor for primary production was fully demonstrated (Schindler 1977), founding the basis for a classical topic in ecosystem empirical modeling (Chapra 1997). The prediction of epilimnetic producers biomass by means of empirical relationships with allochthonous phosphorus is still in use, although some refinements have been developed, including internal loading terms, trophic cascade effects, or organic matter interactions with the light climate (e.g. Beisner and others 2003).

By contrast, the hypolimnion of lakes and reservoirs, although being part of a larger open system, is often considered isolated from direct interactions with the atmosphere and tributaries. In this view, the external drivers can only reach this layer indirectly, through exchanges with the epilimnion, or through interactions with the sediments, which often are modeled as a function of epilimnetic variables (e.g. Carpenter and others 1999). Thus, hypolimnetic quantities or processes like oxygen content or phosphorus internal loading have usually been modeled as a function of variables measured at the epilimnion, or that are supposed to drive epilimnetic processes (Reckhow and Chapra 1983; Nürnberg 1984; Nürnberg 1995; Livingston and Imboden 1996).

For most natural lakes from the temperate and subtropical regions, the assumption that the summer hypolimnion is isolated from direct atmospheric or tributaries interactions appears to be an acceptable simplification. These systems develop a well-defined thermocline that precludes direct exchanges between deep layers and the atmosphere (Wetzel 2001), and the runoff penetration into the hypolimnion is small and dispersive (Wetzel 1990). However, man-made mainstream reservoirs show a relatively high drainage basin area to water body area (BA:WA) ratio (Straškraba 1998), implying a strong advective flux from the river tributaries even during the low-flow summer conditions. This promotes the recurrent presence of the river water as density currents in the reservoir (Ford 1990), which can plunge into the hypolimnion. In such a scenario, the assumption that the hypolimnion is isolated from the direct influence of the river inflow would not be correct.

The empirical modeling of the oxygen content or oxygen deficit in the hypolimnion of water bodies is a good example of a hypolimnetic quantity modeled as a function of epilimnetic processes. Although both lake morphometry and hydrology are usually present in the empirical equations, the phytoplankton biomass, which is supposed to eventually sink and decay in the deep layers, always has a preeminent role. The epilimnetic productivity can be incorporated in the equations using different proxies, including photosynthetic pigments concentration (Charlton 1980), in-lake phosphorus content (Cornett and Rigler 1980; Molot and others 1992), phosphorus retention (Cornett and Rigler 1979; Cornett 1989), or the phosphorus river content (Reckhow 1979; Nürnberg 1995). However, with the exception of brown-water lakes (Hutchinson 1957; Houser and others 2004), and lakes receiving significant humic and fulvic acids inputs (Nürnberg 1995), no significant role on the hypolimnetic oxygen consumption has typically been assigned to the input of allochthonous oxidable substances. Lately, several studies have highlighted the importance of allochthonous organic mater for the oxygen consumption and the metabolic balance in oligotrophic lakes (del Giorgio and others 1999; McManus and others 2003; Pace and Prairie 2005), and for bacterial growth (Jansson and others 2003; Kritzberg and others 2004), indicating that the classical nutrient load-response model assumptions may not be applicable to a considerable number of lakes. In spite of this, empirical hypolimnetic oxygen equations based exclusively on morpho-hydrological features and primary productivity are still in use, although the significance test for the effect of the productivity proxy occasionally fails, even in very productive systems (e.g. Nürnberg 2002). In addition, the lability of allochthonous organic mater has been shown to be important in predicting productivity and structure of planktonic communities in lakes (Søndergaard and others 1995; Foreman and Covert 2003), and could be a critical feature to explain the hypolimnetic oxygen content in reservoirs. These systems tend to show relatively short water residence time (weeks to months), and only dissolved materials that decay in a time scale equal or shorter than that of the water retention time will be relevant for carbon and oxygen cycling.

In this work we show that assumptions of classical models concerning the oxygen content in the hypolimnion do not necessarily hold in reservoirs, where the role of allochthonous OM on the hypolimnetic oxygen content should be enhanced by the fact that density currents can directly inject allochthonous materials into the bottom layers. Thus, the OM input bypasses the epilimnetic food web,

which can mask its role in the subsequent hypolimnetic oxygen depletion caused by sedimentation of epilimnetic OM. Following McManus and others (2003) and Biddanda and Cotner (2002), who found that dissolved organic carbon (DOC) from allochthonous sources plays a significant role in the carbon cycling in lakes Superior and Michigan, we hypothesized that allochthonous DOC could exert a significant effect on the hypolimnetic oxygen content even in very productive systems if the DOC input were high enough. A secondary related hypothesis was that the effect of allochthonous DOC on hypolimnetic oxygen content in these rapidly flushed systems should be modulated by the lability of incoming materials.

We applied three different approximations to test these hypotheses. First, we tested for the role of allochthonous DOC in the hypolimnetic oxygen content using long-term data from two reservoirs receiving a relatively high DOC input of human origin. We calculated correlations between the summer hypolimnetic oxygen concentration and several quantities usually involved in empirical formulations of the oxygen content in lakes and reservoirs. To support potential conclusions drawn from this crude approximation, in the second part of our study we compared the summer riverine DOC load with the oxygen consumption in the hypolimnion of these two reservoirs. If the DOC content of the river entering the reservoir controlled the oxygen consumption in the hypolimnetic layer, a good agreement between the river DOC load and the oxygen consumption in the hypolimnion during a given period would exist. Finally, we examined the implications of our results for the empirical modeling of hypolimnetic reservoir anoxia, applying the Nürnberg's (1995) seminal approach on the anoxic factor to the former and two additional reservoirs showing contrasted human impact.

Additionally, since DOC lability characterization is not frequent in databases, we first explored the adequacy of river chloride concentration (much more frequent in databases) as a proxy for the C:N ratio of the OM from reservoir tributaries included in this study. Although the C:N ratio is not a direct measure of bioavailability, it is frequently related to the growth rate of bacteria growing on riverine OM (Sun and others 1997), and to the lability of OM in soils and rivers (Hunt and others 2000; Kalbitz and others 2000; Stepanauskas and others 2002). The rationale for relating chloride to OM lability is that one of the main impacts of urban areas and farming activities on river water quality is the loading of labile organic substances to the watercourses (Viessman and Hammer 1993; Søndergaard and

Middelboe 1995; Westerhoff and Anning 2000; Tchobanoglous and others 2003). Since these human activities are usually associated to inputs of chloride (Daniel and others 2002), we can use chloride concentration as a proxy of the relative impact of these human activities on rivers, and as a rough approximation to C:N ratio and lability of OM.

#### **MATERIALS AND METHODS**

#### **Study sites**

Table 1 summarizes some limnological features of the four reservoirs included in this study. Sau and Foix reservoirs are located in populated areas in NE Spain near the city of Barcelona, and dam the Ter River and the Foix River, respectively. Brownlee (Snake River, Idaho) and Pueblo (Arkansas River, Colorado) reservoirs are located in low population density areas in the USA. This set covers very small reservoirs (Foix) and large reservoirs draining vast areas (Brownlee). However, all of them have water retention times below one year, suggesting that they are rapidly flushed systems. This is not at odds with the high BA:WA ratio showed by the four reservoirs.

Trophic status measured from chlorophyll *a* levels (Chl-a, Table 1) ranges from mesotrophy (Pueblo Reservoir) to hypereutrophy (Foix Reservoir). The human impact in the eutrophication process of Sau (Vidal and Om 1993), Foix (Marcé and others 2000), and Brownlee reservoirs (Freshwater Research and Brown & Caldwell 2001; IDEQ and ODEQ 2004) is well documented. Pueblo Reservoir drains a mountainous region where the human impact seems to be comparatively less important (Crowfoot and others 2003). The average DOC concentration in the river tributaries (Table 1) ranges from 2.5 mg C L<sup>-1</sup> (Arkansas River) to 6.3 mg C L<sup>-1</sup> (Foix River), but little information is available about the bioreactivity of DOC in these rivers.

#### **Data collection**

Data for Sau and Foix reservoirs and their tributaries used in this paper comes from water quality monitoring programs led by the authors. Monitoring of Sau Reservoir started in 1995 and this work includes data until 2005. Sampling was monthly, consisting in surface to bottom 1 m resolution vertical profiles of temperature, pH, conductivity, and oxygen concentration collected with a multiparametric probe (TURO T-611). The sampling station was located at the lacustrine section of the reservoir. Based on these profiles, a variable number of water samples were collected with a 5 L hydrographic bottle (UWITEC), in order to accurately describe the vertical heterogeneity at the sampling point. The samples were stored in dark bottles and immediately processed in a nearby laboratory. Samples were analyzed for total nitrogen (TN) and total phosphorus (TP) by alkaline persulfate oxidation following Grasshoff and others (1983); for particulate carbon by elemental analysis (EA 1108 CHNS-O Carlo Erba); for soluble reactive phosphorus (SRP) by colorimetric methods (Murphy and Riley 1962); for ammonia (Solorzano 1969) and nitrite (Grasshoff and others 1983) also by colorimetric methods; for DOC by a total carbon analyzer (Shimadzu TOC-5000); for chloride (Cl<sup>-</sup>) and nitrate by liquid chromatography (Konik KNK 500-A); and for Chl-a by the trichromatic method (Jeffrey and Humphrey 1975). The sampling at the river location was identical, but consisted only in one integrated water sample collected just upstream the reservoir. However, Chl-a analyses were not performed on river samples. Water leaving the reservoir through deep outlets was also sampled with the same periodicity. The government water agency (Agència Catalana de l'Aigua, ACA) supplied daily hydrological data.

The database for Foix Reservoir is less complete, starting during 1995 but lasting only until 2002, with a one-year gap in 2000. Sampling was more irregular, with bimonthly frequency during some periods. In addition, not all the variables mentioned above for Sau Reservoir were consistently measured throughout the monitoring program in Foix Reservoir, and conspicuous gaps are present for some variables. Lab analyses and field procedures were as detailed above for Sau Reservoir.

Data for the Snake River and for Pueblo Reservoir and its tributary come from the United States Geological Survey National Information System (USGS-NWIS) database. Data for the Snake River were obtained from stations Snake River at Weiser (USGS-NWIS Station Number 13269000), and Snake River near Murphy (USGS-NWIS Station Number 13172500). Collected data included daily streamflow, river Cl<sup>-</sup> concentration, river DOC concentration, total organic carbon, and total organic nitrogen. Daily streamflow, and river Cl<sup>-</sup>, DOC, total organic carbon, and total organic nitrogen concentration for the Arkansas River upstream Pueblo Reservoir were collected at Arkansas River near Portland, CO (USGS-NWIS Station Number 07099200), and at Arkansas River at Portland, CO

(USGS-NWIS Station Number 07097000). AF's for 21 years (1985 to 2005) were calculated as detailed below using data from Pueblo Reservoir Site 7B (USGS-NWIS Station Number 381602104435200), including temperature and dissolved oxygen profiles. However, no useful data for this study collected at Brownlee Reservoir were found in the USGS-NWIS. Alternatively, reservoir data comes from Nürnberg (2002) and Freshwater Research and Brown & Caldwell (2001), consisting in calculations of the AF for seven years (1970, 1991, 1993, 1994, 1997, 1999, and 2000).

#### Chloride as a proxy for human impact and C:N ratio

To use chloride as a tracer of relative human impact we first estimated the chloride concentration in the rivers included in this study as if they were free of human related spills (Cl<sup>-</sup><sub>PRISTINE</sub>). Then, we compared Cl<sup>-</sup><sub>PRISTINE</sub> with the actual concentration measured in the river.

To calculate Cl<sup>-</sup><sub>PRISTINE</sub> we considered the potential natural sources of chloride, mainly weathering and atmospheric inputs. Since none of the four watersheds considered in this study have significant geological sources of chloride [see Whittemore (2000) for Pueblo watershed; ICC (2002) for Sau and Foix watersheds; and Bond (1978) for Brownlee watershed], we neglected the role of geology as a source of this ion. As a result, the background natural Cl<sup>-</sup> concentration will mainly depend on Cl<sup>-</sup> in precipitation. However, chloride concentration in a river free of human related spills (Cl<sup>-</sup><sub>PRISTINE</sub>) could be much higher than chloride concentration in rain, because evapotranspiration concentrates this ion in soils (Lovett and others 2005). Thus, we calculated a crude estimation of Cl<sup>-</sup><sub>PRISTINE</sub> as the quotient between mean chloride concentration in rain and the annual runoff coefficient (i.e., the fraction of precipitation that is eventually delivered as effective runoff to rivers). Annual runoff coefficients were empirically estimated using long term means of precipitation falling on each watershed and the measured streamflow series. Since water residence time in soils is usually greater than one year (Michel 1992), and chloride is not a true conservative ion in watersheds (Lovett and others 2005), reported Cl<sup>-</sup> PRISTINE estimates in this study should be considered as order-of-magnitude annual mean expectations.

The relationship between measured river Cl<sup>-</sup> concentration and the C:N ratio of the organic matter transported by the rivers were empirically assessed with Cl<sup>-</sup> versus C:N correlations tested with the nonparametric Spearman's  $\tau$  test (Sokal and Rolf 1995). Correlations were performed on pooled

data for the four tributaries considered in this study at different time intervals (instantaneous data, annual averages, and long term, i.e. averaging all available data). In Ter and Foix rivers, C:N ratios were calculated from elemental analyses to which inorganic fractions were subtracted. Data for Snake and Arkansas rivers comes from the USGS-NWIS database, measured as total organic carbon and nitrogen (see Data Collection). Particulate and dissolved organic matter fractions were pooled in this analysis.

#### Empirical regressions with hypolimnetic oxygen

In this analysis we only used data from Sau and Foix reservoirs, because databases for Brownlee and Pueblo did not permit a comparable analysis.

The depth of the seasonal thermocline was estimated with the location of a density gradient of at least 0.15 kg m<sup>-3</sup> m<sup>-1</sup>, threshold that consistently avoided the daily thermocline or other transient structures. Then, we calculated volume-weighted concentrations of the different constituents in the epilimnion and hypolimnion, averaging the values obtained between June and September to get mean summer values for each different year. For the epilimnion, mean spring concentrations (March to May) were also calculated. To account for the variability in the reservoir volume, we expressed the variables measured in the hypolimnion and epilimnion as volume-normalized concentrations, (Stålnacke and others 1999):

Normalized concentration<sub>i</sub> = 
$$\frac{Actual \ concentration_i}{V'} \times V_i$$
 (1)

where *Actual concentration* is the mean volume-weighted concentration during the period (summer or spring) of the  $i^{th}$  year,  $V_i$  is the volume of the hypolimnion or epilimnion during the period of the  $i^{th}$  year, and V' is the mean volume of the hypolimnion or epilimnion for the whole period (i.e, all the years in the database).

Mean summer concentrations for variables measured in the river tributaries were computed by reconstructing daily concentration traces using the methodologies described in Marcé and others (2004), and averaging the values obtained between June and September.

Since most classical empirical approximations to the oxygen content in the hypolimnion consider epilimnetic processes, we tested volume-normalized mean summer oxygen concentration in the hypolimnion (O<sub>2</sub><sup>hypo</sup>) against six variables measured during summer in the epilimnion (TP<sub>epi</sub>, SRP<sub>epi</sub>, TN<sub>epi</sub>, DOC<sub>epi</sub>, Cl<sup>-</sup><sub>epi</sub>, and Chl-a<sub>epi</sub>). Since it can be argued that maximum epilimnetic biomass development in temperate water bodies is usually found during the spring, we also tested O<sub>2</sub><sup>hypo</sup> against the mean epilimnetic concentrations calculated for the spring periods (Spring TP<sub>epi</sub>, Spring SRP<sub>epi</sub>, Spring TN<sub>epi</sub>, Spring DOC<sub>epi</sub>, Spring Cl<sup>-</sup><sub>epi</sub>, and Spring Chl-a<sub>epi</sub>). Finally, we calculated correlations between O<sub>2</sub><sup>hypo</sup> and variables measured during summer in the river inflow (TP<sub>inflow</sub>, SRP<sub>inflow</sub>, TN<sub>inflow</sub>, DOC<sub>inflow</sub>, and Cl<sup>-</sup><sub>inflow</sub>), and also with the mean water inflow to the reservoir during summer.

Correlations were tested using the Spearman's  $\tau$  test, because the number of available observations (maximum n = 11) did not allow an accurate assessment of assumptions needed to apply parametric procedures. In addition, due to the large number of significance tests performed for each reservoir (i.e., 18), we recalculated all significance levels applying the sequential Bonferroni adjustment (Sokal and Rolf 1995), to account for the increased probability of finding spurious significant results.

#### Oxygen consumption in the hypolimnion as a first order process

We compared the hypolimnetic oxygen consumption with the allochthonous DOC load in monthly or bimonthly periods during summer (June to September) in Sau and Foix reservoirs. We accounted for the difference in the oxygen content of the hypolimnetic layer between the start and the end of the period ( $\Delta O_{2hypo} = O_{2hypo_{initial}} - O_{2hypo_{final}}$ ), the oxygen load from the river inflow during the same period ( $O_{2inflow}$ ), and the oxygen leaving the hypolimnion through deep outlets ( $O_{2out}$ ):

Oxygen consumption 
$$(mol O_2 \ period^{-1}) = \Delta O_{2hypo} + O_{2inflow} - O_{2out}$$
 (2)

 $\Delta O_{2hypo}$  was calculated from the vertical oxygen profiles and the volume of water stored in the hypolimnion.  $O_{2inflow}$  was calculated from the daily water inflow and the oxygen data measured upstream the reservoir.  $O_{2out}$  was calculated by interpolating the oxygen concentration measured in the water leaving the reservoir, to build a daily series that was combined with the daily outflow record. To

guarantee that advective or turbulent exchanges between epilimnion and hypolimnion were not severely influencing this analysis, periods showing final oxygen concentration higher than the initial one were discarded, because increasing oxygen concentrations in the bottom layers could be the result of strong mixing with surface water. Noticeably, most discarded periods corresponded to the beginning or the end of the summer, when mixing episodes are more probable. We found 28 valid periods for Sau Reservoir, but only three valid periods could be extracted from the Foix Reservoir database. The DOC load from the river inflow for the same periods (mol C period<sup>-1</sup>) was calculated following Marcé and others (2004) from measured river DOC concentrations and the daily streamflow record.

The above analysis assumes that most of the river inflow directly enters the hypolimnion via a density current during summer, and that river water effectively mixes with hypolimnetic water. To assess whether this was a valid assumption, we estimated the river insertion depth from the temperature profiles collected in the reservoirs, and the temperature measured in the rivers. Although some aspects of the river circulation in a lentic ecosystem are difficult to simplify, the depth of intrusion of river water in the reservoir can be reasonably well predicted assuming that the river inflow will plunge to the depth that minimizes the density differences between river and reservoir water (Armengol and others 1999). At least in Sau Reservoir the adequacy of this approach and the effective mixing of the river water with the hypolimnion once the river plunges into it has been confirmed with empirical work, using conductivity as a tracer of river water and Doppler current profiles measurements (Vidal 2006), and also with hydrodynamic modelling (Rueda and others 2006).

#### **Anoxic Factor modeling**

In the seminal paper by Nürnberg (1995), the hypolimnetic oxygen content was described by means of the anoxic factor (AF, days season<sup>-1</sup>), a variable that accounts for the spatial and temporal extent of the anoxia and is therefore comparable between systems or between periods in a single water body. AF is given by:

$$AF = \sum_{i=1}^{n} \frac{t_i \times a_i}{A_0} \tag{3}$$

where  $t_i$  stands for the period (days) of anoxia (i.e. water with less than 1 mg O<sub>2</sub> L<sup>-1</sup>),  $a_i$  the corresponding anoxic area (m<sup>2</sup>),  $A_0$  the average surface lake or reservoir area during the period (m<sup>2</sup>), and n is the number of periods with different oxycline depths. Since it was first proposed, the AF has been widely used as a tool for lake and reservoir management (Nürnberg 2002; Townsend 1999; Matthews and others 2006), and as a descriptor of eutrophication trends in paleolimnological research (Quinlan and others 1998; Reavie 2006).

In Sau, Foix, and Pueblo reservoirs the summer AF (June to September) was calculated from the oxygen profiles. The exact depth of the 1 mg  $O_2 L^{-1}$  isopleth was estimated by linear interpolation, and the corresponding area was calculated with reservoir-specific depth-area plots. Anoxic area values between sampling dates were linearly interpolated. For Brownlee Reservoir we used the AF values calculated in Nürnberg (2002).

Empirical regressions between the AF and variables usually involved in the prediction of this variable were then calculated. Since some measure of the hydrological behavior of the system is always considered in the equations (Townsend 1999; Nürnberg 2002), we included the mean summer inflow as a master independent variable. In addition, classical proxies of the primary production in the epilimnion (TP<sub>epi</sub>, Chl-a<sub>epi</sub>, Spring TP<sub>epi</sub>, and Spring Chl-a<sub>epi</sub>) were combined with the mean summer inflow in multiple regressions with two independent variables. Finally, DOC<sub>inflow</sub> and Cl<sup>-</sup><sub>inflow</sub> were also included as independent variables to assess the effect of the inflowing materials on the AF interannual variability. Due to limitations in the available data, regressions with the epilimnetic variables and DOC<sub>inflow</sub> were not calculated for Brownlee Reservoir, and only Chl-a<sub>epi</sub> was available in Pueblo Reservoir. For the same reason, some of the AF calculations could not be used because we could not calculate appropriate paired data from the river. Finally, since few cases were generally available to calculate regressions, the normality assumption could not be correctly assessed, although extreme outliers were not present in any variable. Consequently, all significance levels (for both the whole regression and the partial effects) were calculated with permutation tests (Anderson and Legendre 1999), using software by Legendre (2002).

#### RESULTS

#### Chloride as a proxy for human impact and C:N ratio

Average observed river chloride concentrations ranged nearly two orders of magnitude, with higher values in Foix and Ter rivers than in Snake and Arkansas (Table 1). These river Cl<sup>-</sup> concentrations were much higher than the mean chloride concentration measured in rain falling on the watersheds drained by the rivers (Table 1). Estimated Cl<sup>-</sup><sub>PRISTINE</sub> values were very similar in Ter, Snake, and Arkansas rivers (between 3 and 5 mg L<sup>-1</sup>, Table 1), while in Foix they were much higher, probably due to its proximity to the sea (6 km). Remarkably, the value estimated for the Ter River coincided reasonably well with mean chloride concentrations measured in pristine streams near the sampling point [4 to 7 mg L<sup>-1</sup>, Butturini (1998)], and values for the Foix River were very similar to mean chloride concentration measured in pristine streams of the same typology in the area [17 to 28 mg L<sup>-1</sup>, Bernal (2006)]. The comparison between observed river Cl<sup>-</sup> concentrations and Cl<sup>-</sup><sub>PRISTINE</sub> revealed rather weak differences in the Arkansas River, and only modest differences in the Snake River (Table 1). However, observed Cl<sup>-</sup> was much higher than Cl<sup>-</sup><sub>PRISTINE</sub> in Ter and Foix rivers.

The C:N ratio of the river organic matter showed a logarithmic relationship with observed river Cl<sup>-</sup> (Figure 1). Although nonparametric correlations for instantaneous data, annual means, and log term means were all significant, Spearman's  $\tau$  correlation greatly increased as data were pooled in longer time periods. Remarkably, rivers with high Cl<sup>-</sup> content (Ter and Foix rivers) showed low organic matter C:N ratios, while rivers with low Cl<sup>-</sup> concentrations (Snake and Arkansas) showed high C:N ratios.

#### Allochthonous DOC and hypolimnetic O<sub>2</sub>

The Sau Reservoir database includes almost all the annual inflow range found in the whole history of the reservoir (Armengol and others 1991), and we also found a wide range in the streamflow measured in the Foix River (Figure 2*A*). Thus, both very wet years and dry periods are lumped in subsequent analyses. This is a very important point, because hydrology in the Mediterranean region is highly variable, and this has profound effects on reservoir processes (Geraldes and Boavida 2004).

We can observe a clear trend in TN and DOC concentrations in the inflow to Sau Reservoir, which decreased by almost 50% during the eleven years period (Figure 2C and D). This has been

related to the implementation of biological treatments in the wastewater treatment plants of the watershed during the late 1990's (Marcé and others 2006), because these treatments are efficient in removing nitrogen compounds and OM from effluents. By contrast, TP concentration did not follow a clear trend (Figure 2*B*), because very high river TP concentrations measured in the river were already lowered by the physicochemical treatments implemented during the late 1980's (Marcé and others 2004). Time series for the Foix River are less complete, and it is difficult to establish temporal trends. However, it is worth mentioning the extremely high TN and TP concentrations found in this reservoir inflow.

In Sau Reservoir the classical proxies of the epilimnetic algal biomass (TP and Chl-a, Figure 2E) did not respond to the temporal changes of nutrient inputs from the river. However,  $O_2^{hypo}$  (Figure 2F) showed a conspicuous increase as nitrogen and DOC in the inflow decreased, suggesting that river TN and river DOC concentrations could play a role in the hypolimnetic oxygen content. In the case of Foix Reservoir, the evolution of the oxygen content in the hypolimnion did not follow the temporal trend of TP or TN concentration in the river inflow. In any case, although the proxies for the epilimnetic algal biomass showed huge variability in both reservoirs, epilimnetic biomass did not seem to play a principal role in shaping the hypolimnetic oxygen content in these reservoirs.

During the empirical regression analysis, no variable measured in the epilimnion during summer or spring gave significant results when tested against the oxygen content of the hypolimnion (Table 2). By contrast, only variables measured in the river inflow were significantly correlated with  $O_2^{hypo}$ . Remarkably, in both reservoirs  $DOC_{inflow}$  and  $Cl_{inflow}$  where strongly correlated with  $O_2^{hypo}$  (Table 2). However, only three data points for  $DOC_{inflow}$  were available for Foix Reservoir, and interpretations should be cautious in this case. In Sau Reservoir  $O_2^{hypo}$  also correlated with  $TN_{inflow}$ . The significant relationships in Table 2 do not seem to be inflated by hydrology. The summer inflow was not significantly correlated with  $O_2^{hypo}$ , and the variables involved in significant correlations did not show significant relationships with summer inflow (Table 3), except  $DOC_{inflow}$  in Foix Reservoir.

The allochthonous DOC and  $O_2^{hypo}$  short term mass balances showed a close agreement in Sau Reservoir (Figure 3). The required assumption of deep intrusion of the river during summer held in both reservoirs (Figure 4), with river inflow directly plunging into the hypolimnion or at the metalimnetic

level as an interflow in most situations. The relationship between river DOC load and oxygen consumption in Sau hypolimnion (Figure 3) was well established in the 1:1 line (*slope* =  $0.92 \pm 0.09$ ,  $r^2$ = 0.81, *P* Value < 0.0001, n = 28). We also found a positive and highly significant relationship between river DOC load and oxygen consumption in the hypolimnion of Foix Reservoir (*slope* =  $0.63 \pm 0.007$ ,  $r^2$ = 0.99, *P* Value = 0.007, n = 3), although in this case the slope significantly departed from one.

#### **AF modeling**

The results from the different regression equations to predict AF in the four reservoirs considered are compiled in Table 4. Remarkably, where data was available to calculate regressions including variables measured in the epilimnion (i.e., Sau, Foix, and Pueblo), we did not find statistical justifications to include these variables in the prediction of AF (see Equations 4 to 7, 10 to 13, and 24 and 28 in Table 4). By contrast, in Sau and Foix reservoirs DOC<sub>inflow</sub> and Cl<sup>-</sup><sub>inflow</sub> were significant variables explaining the year-to-year variability of AF. In Sau Reservoir the combination of both DOC<sub>inflow</sub> and Cl<sup>-</sup><sub>inflow</sub> with the summer inflow was statistically supported (Equations 8 and 9), since the partial effects of the independent variables were significant. In Foix Reservoir the combined effect of summer inflow and DOC<sub>inflow</sub> could not be tested due to the small sample size. But regressions including the summer inflow (Equations 15 and 16) did not support the inclusion of this variable as a significant explaining factor. By contrast, regressions using DOC<sub>inflow</sub> and Cl<sup>-</sup><sub>inflow</sub> showed a very high coefficient of determination (i.e.,  $r^2 > 0.90$ , Equations 17 and 18), although in the first case the significance level was badly affected by the small sample size.

In Brownlee Reservoir the dependence of AF on the combination of  $Cl_{inflow}$  and the summer inflow were not supported by available data (Equation 19 in Table 4), because these variables were correlated (*Spearman's*  $\tau = -0.86$ , *P* Value = 0.014, *n* = 7). This makes it difficult to estimate the partial effects of these variables on AF. However, partial  $r^2$  values for Equation 19, and results from Equations 20 and 21 suggest that  $Cl_{inflow}$  has an important role on AF variability. By contrast, in Pueblo Reservoir the summer water inflow was the only significant variable (Equations 23 and 25, partial significance for this variable in Equations 22 and 24 seems to be badly influenced by the sample size). DOC<sub>inflow</sub> (Equation 26) and  $Cl_{inflow}$  were not considered relevant, despite the significant correlation between Cl<sup>-</sup> <sup>inflow</sup> and AF (Equation 27). Considering partial  $r^2$  values in Equation 23, and the correlation between summer inflow and Cl<sup>-</sup><sub>inflow</sub> (*Spearman's*  $\tau = -0.81$ , *P* Value = 0.003, *n* = 11), results in Equation 27 are mostly attributed to the effect of summer inflow on Cl<sup>-</sup><sub>inflow</sub> values. An interesting result in Equation 24 was the high *F*-Ratio and partial  $r^2$  associated to Chl-a<sub>epi</sub> (the summer inflow and Chl-a<sub>epi</sub> were not correlated, *Spearman's*  $\tau = -0.40$ , *P* Value = 0.600, *n* = 4). Although neither Inflow nor Chl-a<sub>epi</sub> was a significant factor in the regression, the small sample size and the very high whole regression  $r^2$ suggested that with more data on hand AF modeling in Pueblo would be better modeled by combining a hydrological variable with a proxy for epilimnetic productivity.

#### DISCUSSION

Since no geological sources were supposed, observed chloride concentrations in the tributaries much higher than corresponding Cl<sup>-</sup><sub>PRISTINE</sub> (especially in Ter and Foix rivers) must be attributed to human activities (i.e., urban, industrial, and farming activities). Deicing salt road application were not considered relevant for this study, because the chemical effects of road runoff on surface water ecosystems are usually confined to small streams (Forman and Alexander 1998) and highly urbanized watersheds (Kaushal and others 2005), and salt road application in the Spanish watersheds considered in this study is very unusual.

Following the previous reasoning and values in Table 1, we used Cl<sup>-</sup> concentration in river inflows as an estimate of the relative impact of urban areas and farming activities. By extension, this also worked as a rough but useful tracer of the origin of the DOC content of these rivers. In this context, low DOC content in the Arkansas River upstream Pueblo Reservoir seems to be mainly associated to terrestrial, low reactive sources; while most DOC upstream Sau and Foix reservoirs are probably labile DOC coming from human activities. Conclusions for Sau Reservoir were not at odds with the empirical evidence that most of the incoming river DOC is labile, coming mainly from urban, industrial, and farming spills that accumulate upstream the reservoir (Romaní and Sabater 1999). By contrast, high DOC values in the Snake River coincided with relatively low chloride concentrations, suggesting that most of the DOC carried by the Snake River is not of human origin, but comes from terrestrial

ecosystems. The same conclusion was reached by Harrison (2005), who reported that the labile fraction in the Snake River upstream Brownlee Reservoir seems to be a small percentage of the total DOC.

The above interpretations were also supported by the C:N atomic ratio of the OM (Table 1) and its relationship with river chloride concentration (Figure 1). C:N values above 15 indicate strong influence of OM coming from terrestrial ecosystems (Kendall and others 2001; McKnight and others 2003), while values below 4 are exceptional (Kendall and others 2001), and should be attributed to the influence of spills loaded with nitrogen rich organic compounds like urine or slurries (Werner and others 1989). There is also strong evidence that low C:N ratio in the OM is a good indicator of organic carbon bioavailability (Goldman and others 1987; Kroer 1993; Sun and others 1997; Hunt and others 2000; Kalbitz and others 2000; Stepanauskas and others 2002; Benner 2003). Since C:N ratios are affected by in-stream processes and several natural and anthropogenic sources (Findlay and Sinsabaugh 2003), the relationship using instantaneous data (Figure 1A) showed substantial scatter. However, as we lumped data into longer time periods, e.g. annual or long term means (Figures 1B and C), the influence of human activities on the C:N ratio was much crisper. Last but not least,  $\delta^{13}$ C values measured in the particulate matter upstream Sau Reservoir in 2000 (Rafael Marcé, Unpublished Data) ranged between -23.90 and -18.05 ‰, which are substantially higher than typical values for rivers (Kendall and others 2001; Raymond and Bauer 2001; McCallister and others 2004). This also suggests the presence of a supplementary organic carbon source not related to terrestrial ecosystems.

All in all, the four systems included in this study can be placed in a gradient of increasing relative human impact, and of lability of OM coming from tributaries. In this ordination, Foix Reservoir suffers the strongest human impact, and receives most DOC load in labile, nitrogen rich forms. In the opposite side, Pueblo Reservoir undergoes a relatively low human impact, and receives organic carbon in low reactive forms.

Results from Sau and Foix reservoirs clearly showed that the summer hypolimnetic oxygen content in these water bodies is largely controlled by external DOC inputs. In these reservoirs  $DOC_{inflow}$ and  $Cl_{inflow}$  were well correlated with  $O_2^{hypo}$ , suggesting that human derived organic matter could play a principal role in controlling  $O_2^{hypo}$ . The correlation between  $TN_{inflow}$  and  $O_2^{hypo}$  was also significant in Sau Reservoir, but considering the good agreement between DOC load and  $O_2$  consumption in this

reservoir, this significant correlation is most probably a consequence of the collinearity between  $DOC_{inflow}$  and  $TN_{inflow}$  (Figure 2*C* and *D*, and Table 3).

The effect of allochthonous DOC on O<sub>2</sub><sup>hypo</sup> was confirmed by the close relationship found between the river DOC load and the oxygen consumption in the hypolimnion of Sau Reservoir during summer (data for Foix are scarce and not conclusive), strongly supporting our hypothesis that oxidation of riverine DOC controls the oxygen content in the hypolimnion. The rationale of this approach relies on the assumption that the decay of organic carbon is stoichiometrically linked to oxygen consumption. For oxic environments this is a reasonable assumption, since values of the respiratory quotient for most organic materials are close to one (Williams and del Giorgio 2005). However, if other metabolic pathways not involving oxygen as electron acceptor are important for the carbon cycling, the relationship between organic carbon decay and oxygen consumption could be masked. This could be the case in Foix Reservoir, since the slope of the relationship between river DOC load and oxygen consumption in the hypolimnion significantly departed from one, suggesting the presence of important metabolic pathways other than oxygen respiration in this reservoir. However, the extent of the database did not allow to accurately test this supposition.

The significant correlations between oxygen content and river CI<sup>-</sup> (Table 2) support the conclusion that DOC carried by the river inflows in Foix and Sau reservoirs is mainly from human origin. Thus, human derived spills directly controlled the hypolimnetic oxygen content in these reservoirs, and OM produced in the epilimnion did not appear to be a key factor. From a theoretical point of view this has important implications, because despite the fact that the effect of external DOC inputs on hypolimnetic oxygen have been considered in some classical studies (Hutchinson 1957; Nürnberg 1995), virtually all the empirical models considering processes in the hypolimnion are based on the assumption that the key driving process is the decay of OM sinking from the epilimnion. A general advice coming from our results is that assumptions of classical models concerning the oxygen content in the hypolimnion do not necessarily hold in reservoirs under strong human impact.

The above conclusion also reaches other hypolimnetic processes that are essentially dependent on the hypolimnetic oxygen concentration. The internal load of phosphorus is a good example, since it is usually modeled as a function of some measure of lake productivity or of the anoxia extent

(Carpenter and others 1999; Nürnberg and LaZerte 2004). Obviously, if the oxygen concentration is dependent on the external DOC input, internal load of phosphorus will also be dependent on it to some extent. This is very interesting from a management point of view, because it means that in some circumstances one of the key processes governing water quality resilience of temperate water bodies (Folke and others 2004) are linked to a process that could be directly controlled by human intervention in the watershed (i.e. reducing DOC load from human related spills). That is, we can reduce oxygen consumption considering a first order process related to DOC input, without the need to deal with the frequently non-linear, complex relationship between nutrient load reduction, algal biomass response, and concomitant reduction in oxygen consumption. Also, the hypolimnetic oxygen control by external DOC inputs are a possible explanation of some disappointments of lake recovering programs based on nutrient load reduction (see Cooke and others 1993).

Results from the AF modeling suggest that at least in reservoirs the effect of allochthonous DOC input on the hypolimnetic oxygen content depends on the lability of DOC entering the waterbody, which is not at odds with the recurrent observation that bacterial metabolism are not well correlated with bulk dissolved OM (Findlay 2003). This view was supported by the relative importance of hydrology and Cl<sup>-</sup><sub>inflow</sub> on AF prediction (Figure 5). While in the most impaired reservoir (Foix Reservoir, Figure 5A and B) AF variability could exclusively be explained by the proxy of OM lability (i.e. Cl<sup>-</sup>inflow), as we move to reservoirs less influenced by human related spills the importance of allochthonous materials on AF decreases, and the relevance of hydrology increases (Figure 5 and Table 4). That is, as the C:N ratio of the OM entering the reservoir decreases, the relevance of the oxidation of these materials on the hypolimnetic oxygen content increases. This is coherent in reservoirs, because these systems have short water residence times, and only materials that decay in a time scale equal or shorter than that of the water retention time will be relevant for carbon and oxygen cycling. Water retention time in reservoirs is typically below one year, and values less than 100 days are frequently recorded (e.g. Table 1). Considering that only the most labile DOC fractions are consumed in these time scales (del Giorgio and Davis 2003), it is reasonable to predict that in these rapidly flushed systems only the labile DOC fractions will be relevant for carbon and oxygen dynamics. Although our database did not allow to fully demonstrate this hypothesis, results from the AF modeling seem to point in this

direction. Reservoirs receiving OM with low C:N ratios (i.e. Sau and Foix reservoirs) showed important DOC<sub>inflow</sub> effects (Equations 8 and 17), while in the reservoir showing the highest C:N ratio in inflowing OM (Pueblo Reservoir), DOC<sub>inflow</sub> was a non significant factor (Equations 22 and 25). Consequently, a more important role of autochthonous OM on oxygen consumption would be expected in reservoirs where DOC<sub>inflow</sub> is a non significant factor. In fact, Equation 24 suggested a combined effect of hydrology and algal biomass to explain AF in Pueblo Reservoir, although the small sample size did not allow to fully demonstrate this effect.

We could take the assessment of the importance of DOC lability in driving the AF and oxygen depletion a step further by conducting a simple bounding exercise. Since general trends of DOC consumption over time are well established for river water [%DOC consumed at  $20^{\circ}C = 2.27 \times$ Time(days)<sup>0.55</sup>, n = 45, del Giorgio and Davis (2003)], we could calculate the expected fraction of riverine DOC consumed in a reservoir considering its water residence time. Then, we can compare this figure against the bioavailable fraction of riverine DOC needed to generate the observed oxygen depletion in the hypolimnion assuming that all oxygen consumption is due to allochthonous sources. A large departure between these two fractions may be either an indication that DOC lability in the river tributary is far from typical, or an indication that the assumption that oxygen depletion is mainly due to allochthonous sources is flawed. Averaging observed data from the reservoirs placed in the extremes of the gradient of lability of OM coming from tributaries (Sau and Pueblo), we obtained that 82% and 47% of riverine DOC should be consumed to account for the oxygen depletion observed in Sau and Pueblo reservoirs, respectively [these percentages were standardized to 20°C following del Giorgio and Davis (2003)]. Applying the above equation using the water residence time in these reservoirs, expected percent riverine DOC consumption is 31% for Sau and 55% for Pueblo. Taking into account that none of the analyses in this work supported a significant role of autochthonous organic matter on oxygen depletion in Sau Reservoir, the wide difference between the expected DOC consumption if Ter River were an average river (31%) and the actual result (82%) should be attributed to the presence of large quantities of human derived labile DOC in the Ter River. Interestingly, although DOC<sub>inflow</sub> was a significant factor on AF prediction in Sau Reservoir but was not in Pueblo, during an average summer Pueblo receives 0.136 mg DOC day<sup>-1</sup> per liter of water stored in the hypolimnion, while only 0.062 mg

DOC day<sup>-1</sup> L<sup>-1</sup> arrives in Sau Reservoir. Thus, large differences in the biodegradability of DOC should exist to explain the different behavior of the oxygen dynamics in these systems.

The conclusions outlined above make an extensive study of the allochthonous DOC effect on hypolimnetic oxygen in reservoirs desirable. In principle, the effect of external DOC inputs on the hypolimnetic oxygen content of lakes has been already tested by Nürnberg (1995). She concluded that although the external DOC load largely explained the year-to-year variability of AF in single lakes, this variable did not have any significant effect on between lakes AF prediction. However, the last statement was supported by the lack of relationship between DOC load and AF including only a small subset of lakes (*P* Value < 0.32, *n* = 8). Interestingly, if we reevaluate the relationship from which one of the most influential conclusions in Nürnberg's paper were drawn (TP loading against lake AF,  $r^2 = 0.76$ , *P* Value < 0.0001, *n* = 17), but considering only this subset of lakes, the effect of TP loading vanishes ( $r^2$ = 0.005, *P* Value = 0.873, *n* = 8). Our intention is not to diminish the value of Nürnberg's (1995) study, because several analyses in this paper confirmed the importance of TP on AF prediction, and she already noticed the possible effect of DOC load if more samples were available. But in our opinion we still lack a conclusive cross-sectional study on the effect of allochthonous DOC on the oxygen content of lakes and reservoirs.

To conclude, results from this work emphasize that the role of allochthonous inputs in determining hypolimnetic dissolved oxygen concentrations has not received nearly the attention that epilimnetic primary production and TP have received. However, allochthonous DOC can be a very important driver in reservoirs under strong human impact. This should prompt practitioners to test the possible effect of OM entering the system when dealing with impaired waters. Also, the hypolimnion of reservoirs should be regarded as a system potentially opened to their watershed. This should always be considered when formulating empirical models for these systems.

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#### **FIGURE LEGENDS**

**Figure 1**. C:N ratio in organic matter against chloride concentration in the river tributaries of the four reservoirs included in this study. Particulate and dissolved organic matter fractions are pooled in these plots. Spearman's  $\tau$  correlation and significance using **A** instantaneous data, **B** annual averages, and **C** long term averages are detailed. Panel **B** only includes years with at least three data pairs to calculate the annual mean. Symbols in the different panels are as defined in panel **C**. Dotted horizontals lines at C:N = 15 and C:N = 4 and logarithmic regressions (thick lines) are plotted for reference.

**Figure 2**. **A** Annual flow and box-whisker plots for measured **B** TP, **C** TN, and **D** DOC concentration in the Ter River (Sau Reservoir tributary, *left*), and in the Foix River (Foix Reservoir tributary, *right*). Time trace of **E** epilimnetic TP and Chl-a concentrations, and **F** hypolimnetic oxygen content in Sau Reservoir (*left*), and Foix Reservoir (*right*). Concentrations in panels **E** and **F** are volume-normalized.

**Figure 3**. Estimated oxygen consumption in Sau Reservoir hypolimnion against DOC load from the Ter River in monthly or bimonthly periods.

**Figure 4**. Thermocline and river insertion depth for an average year in Sau Reservoir (*left*) and Foix Reservoir (*right*).

**Figure 5**. AF predictions (regression line and 95% confidence intervals) in (**A**, **B**) Foix Reservoir, (**C**, **D**) Sau Reservoir, (**E**, **F**) Brownlee Reservoir, and (**G**,**H**) Pueblo Reservoir. Equation numbers refer to equations in Table 4. Independent variables included in the equation are given in brackets at the bottom of each panel, and the symbol ? denotes a non-significant regression. Panels were arranged to place reservoirs in a gradient of relative human impact (Foix Reservoir at the top, Pueblo Reservoir at the bottom).









Month of a prototype year



#### **TABLE LEGENDS**

**Table 1**. Selected limnological features of the four reservoirs and tributaries included in this study. For

 some magnitudes standard deviation are given in brackets.

**Table 2**. Spearman's  $\tau$  correlation of volume-normalized mean summer oxygen concentration in the hypolimnion of Sau and Foix reservoirs against selected independent variables (see text for details). All significance levels are Bonferroni corrected *P* Values.

**Table 3**. Spearman's  $\tau$  correlation between independent variables involved in significant correlations in Table 2, and the summer inflow. The upper side of both correlation matrices contains the sample size in brackets.

 Table 4. AF modeling results for the four reservoirs considered. All significance levels in this table

 were calculated with permutation tests. Significance levels below 0.05 are highlighted for clarity.

 Equation 14 was included to avoid confusions.

	<b>Sau</b> (Ter River) <sup>a</sup>	<b>Foix</b> (Foix River) <sup>b</sup>	<b>Brownlee</b> (Snake River) <sup>c</sup>	<b>Pueblo</b> (Arkansas River) <sup>d</sup>
Maximum volume (hm <sup>3</sup> )	168.5	5.6	1533	650.8
Maximum depth (m)	65	10	85	52
Maximum area (WA, km <sup>2</sup> )	5.8	0.72	47.5	32.3
Basin area (BA, km <sup>2</sup> )	1380	289	189000	23906
BA:WA ratio	238	401	3981	740
Mean inflow (hm <sup>3</sup> yr <sup>-1</sup> )	540.5	19.3	15632	713.4
Mean residence time (yr)	0.31	0.29	0.08	0.91
Mean Chl-a in epilimnion ( $\mu g L^{-1}$ )	9.6	95.8	7.0 <sup>e</sup>	3.9
DOC in river inflow (mg L <sup>-1</sup> )	4.1 (2.3)	6.3 (1.4)	4.5 (3.1)	2.5 (0.4)
C:N ratio in river organic matter	8.3 (6.5)	3.1 (2.4)	12.5 (14.0)	17.8 (12.1)
Chloride in river inflow (mg L <sup>-1</sup> )	94.9 (53.2)	238.6 (107.4)	19.7 (5.5)	8.2 (3.5)
Mean chloride in rain (mg L <sup>-1</sup> )	1.01 <sup>f</sup>	1.90 <sup>g</sup>	0.68 <sup>h</sup>	0.35 <sup>i</sup>
Annual watershed runoff coefficient	0.32	0.07	0.20	0.08
Cl <sup>-</sup> <sub>PRISTINE</sub> in river inflow (mg L <sup>-1</sup> )	3.17	25.86	3.48	4.52

<sup>a</sup> For DOC data n = 129, for chloride data n = 132, and for C:N ratio n = 103.

<sup>b</sup> For DOC data n = 21, for chloride data n = 27, and for C:N ratio n = 12.

<sup>°</sup>For DOC data n = 171, for chloride data n = 166, and for C:N ratio n = 124.

<sup>d</sup> For DOC data n = 23, for chloride data n = 181, and for C:N ratio n = 8.

<sup>e</sup> Freshwater Research and Brown & Caldwell (2001). Data are for Site 5 in this report.

<sup>f</sup> Avila and Alarcon (1999).

<sup>g</sup> Alcalá (2005).

<sup>h</sup> James P. McNamara (Unpublished Data).

<sup>i</sup> Claassen and Halm (1995).

	Sa	au Reservoir	F	'oix Reservoir
	n	Spearman's τ	n	Spearman's t
Summer inflow	11	-0.08 <sup>ns</sup>	7	0.71 <sup>ns</sup>
TP <sub>epi</sub>	11	-0.29 <sup>ns</sup>	6	0.20 <sup>ns</sup>
SRP <sub>epi</sub>	11	-0.35 <sup>ns</sup>	7	0.50 <sup>ns</sup>
TN <sub>epi</sub>	11	-0.23 <sup>ns</sup>	7	0.71 <sup>ns</sup>
DOC <sub>epi</sub>	10	-0.14 <sup>ns</sup>	3	-0.50 <sup>ns</sup>
Chl-a <sub>epi</sub>	11	-0.54 <sup>ns</sup>	7	0.64 <sup>ns</sup>
Cl <sup>-</sup> <sub>epi</sub>	11	-0.51 <sup>ns</sup>	7	-0.14 <sup>ns</sup>
Spring TP <sub>epi</sub>	11	0.48 <sup>ns</sup>	7	0.20 <sup>ns</sup>
Spring SRP <sub>epi</sub>	11	0.30 <sup>ns</sup>	7	0.42 <sup>ns</sup>
Spring TN <sub>epi</sub>	11	0.01 <sup>ns</sup>	7	0.64 <sup>ns</sup>
Spring DOC <sub>epi</sub>	10	0.16 <sup>ns</sup>	5	-0.32 <sup>ns</sup>
Spring Chl-a <sub>epi</sub>	11	0.35 <sup>ns</sup>	7	-0.13 <sup>ns</sup>
Spring Cl <sup>-</sup> <sub>epi</sub>	11	-0.31 <sup>ns</sup>	7	0.27 <sup>ns</sup>
$TP_{inflow}$	11	-0.35 <sup>ns</sup>	6	0.26 <sup>ns</sup>
$SRP_{inflow}$	11	-0.31 <sup>ns</sup>	7	-0.29 <sup>ns</sup>
$TN_{inflow}$	11	-0.89 **	7	0.39 <sup>ns</sup>
DOC <sub>inflow</sub>	11	-0.81*	3	-1.00 ***
Cl <sup>-</sup> inflow	11	-0.87 **	7	-1.00 ***

Bonferroni adjusted significance levels: ns, non significant; \* *P* Value < 0.05; \*\* *P* Value < 0.01; \*\*\* *P* Value < 0.001.

	Summer inflow	$TN_{\text{inflow}}$	$DOC_{\text{inflow}}$	$Cl_{inflow}$
Sau				
Summer inflow	-	(11)	(11)	(11)
$TN_{inflow}$	0.245 <sup>ns</sup>	-	(11)	(11)
DOC <sub>inflow</sub>	0.373 <sup>ns</sup>	0.936 ***	-	(11)
Clinflow	-0.245 <sup>ns</sup>	0.736 **	0.645 *	-
Foix				
Summer inflow	-	-	(3)	(7)
$TN_{inflow}$	-	-	-	-
DOC <sub>inflow</sub>	-1.000 ***	-	-	(3)
Cl <sup>-</sup> <sub>inflow</sub>	-0.714 <sup>ns</sup>	-	1.000 ***	-

ns, non significant. \* *P* Value < 0.05. \*\* *P* Value < 0.01. \*\*\* *P* Value < 0.001.

Equation			Whole regression		First independent variable			Second independent variable		
index	Regression	n	$R^2$ or $r^2$	P Value	F-Ratio	P Value	Partial $r^2$	F-Ratio	P Value	Partial $r^2$
Sau										
4	$AF = 100.15 - 48.93$ Inflow + 5.12 $TP_{epi}$	11	0.40	0.128	4.98	0.070	0.38	1.10	0.360	0.12
5	AF = 98.37 - 40.72 Inflow + 0.11 Chl-a <sub>epi</sub>	11	0.34	0.175	3.45	0.109	0.30	0.27	0.674	0.03
6	$AF = 97.33 - 33.45$ Inflow - 0.97 Spring $TP_{epi}$	11	0.32	0.216	3.65	0.093	0.31	0.01	0.894	0.00
7	AF = 118.60 - 34.80 Inflow - 2.05 Spring Chl-a <sub>epi</sub>	11	0.50	0.059	5.37	0.061	0.40	2.85	0.126	0.26
8	$AF = 52.22 - 57.37 \text{ Inflow} + 0.18 \text{ DOC}_{inflow}$	11	0.85	0.002	37.48	0.001	0.82	28.03	0.002	0.78
9	$AF = 32.33 - 26.11 \text{ Inflow} + 0.61 \text{ Cl}_{inflow}$	11	0.82	0.002	8.15	0.032	0.50	22.11	0.003	0.73
Foix										
10	$AF = 79.59 - 64.34 \text{ Inflow} - 0.67 \text{ TP}_{epi}$	5	0.50	0.497	1.02	0.374	0.34	0.08	0.813	0.04
11	$AF = 74.18 - 10.37 \text{ Inflow} - 0.13 \text{ Chl-a}_{eni}$	6	0.41	0.272	0.22	0.128	0.11	0.36	0.291	0.07
12	$AF = 64.08 - 21.84 \text{ Inflow} + 0.35 \text{ Spring TP}_{epi}$	6	0.35	0.546	1.53	0.280	0.33	0.07	0.800	0.02
13	AF = 63.77 - 20.33 Inflow + 0.06 Spring Chl-a <sub>epi</sub>	6	0.39	0.540	1.73	0.292	0.37	0.23	0.822	0.07
14	$AF = 105.74 - 176.49 Inflow - 2.79 DOC_{inflow}$	3	-	-	-	-	-	-	-	-
15	$AF = 32.97 - 0.77 \text{ Inflow} + 0.11 \text{ Cl}_{inflow}$	6	0.91	0.021	0.01	0.922	0.00	18.82	0.025	0.86
16	AF = 67.75 - 19.81 Inflow	6	0.34	0.175	-	-	-	-	-	-
17	$AF = -21.36 + 11.41 \text{ DOC}_{inflow}$	3	0.98	0.177	-	-	-	-	-	-
18	$AF = 32.34 + 0.12 Cl_{inflow}$	6	0.91	0.011	-	-	-	-	-	-
Brownlee										
19	$AF = 47.52 - 0.0006 \text{ Inflow} + 2.23 \text{ Cl}_{inflow}$	7	0.80	0.049	0.26	0.654	0.06	2.82	0.150	0.41
20	AF = 121.34 - 0.0020 Inflow	7	0.65	0.031	-	-	-	-	-	-
21	$AF = 26.34 + 2.81 \text{ Cl}_{inflow}$	7	0.78	0.010	-	-	-	-	-	-
Pueblo										
22	$AF = 54.38 - 0.001 \text{ Inflow} + 12.9 \text{ DOC}_{inflow}$	5	0.77	0.147	6.56	0.061	0.77	0.21	0.739	0.09
23	$AF = 25.18 - 0.011 \text{ Inflow} + 1.37 \text{ Cl}_{inflow}$	11	0.80	0.001	12.97	0.008	0.62	0.32	0.587	0.04
24	$AF = 56.85 - 0.052 \text{ Inflow} + 3.48 \text{ Chl-}a_{epi}$	4	0.95	0.154	10.61	0.254	0.91	6.34	0.214	0.86
25	AF = 33.40 - 0.012 Inflow	20	0.74	0.001	-	-	-	-	-	-
26	$AF = 53.11 - 11.820 \text{ DOC}_{inflow}$	6	0.23	0.360	-	-	-	-	-	-
27	$AF = -19.59 + 7.590 Cl_{inflow}$	11	0.48	0.024	-	-	-	-	-	-
28	$AF = 28.52 - 0.921 \text{ Chl-}a_{epi}$	5	0.01	0.843	-	-	-	-	-	-