

Contrasting pelagic plankton in temperate Irish lakes: the relative contribution of hetero-, mixo- and autotrophic components, and the effects of extreme rainfall events

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## **Abstract**

The mobilisation of energy from allochthonous carbon by heterotrophic bacterioplankton can be proportionally more important than autotrophic production in humic lakes. Moreover, increasing levels of dissolved organic carbon (DOC) in many aquatic systems linked to increases in precipitation, which in turn may be related to changing climate, means that this heterotrophic component of the foodweb may play an increasing role in the overall transfer and production of energy, particularly within peatland catchments. While such catchments are very common in the temperate northwest Atlantic regions of Europe, studies describing the seasonal dynamics of the heterotrophic, mixotrophic and autotrophic components of their aquatic foodwebs are rare. In this study, the biomass of these pelagic components was enumerated over one year in two oligotrophic lakes, both situated in peatland catchments in the west of Ireland, but with contrasting DOC concentrations. Bacterial biomass dominated the pelagic foodweb of the more humic lake, Lough Feeagh, while autotrophic phytoplankton biomass was greatest in the clearwater lake, Lough Guitane. The biomass of potentially mixotrophic flagellates was also slightly larger in the Lough Guitane, while phagotrophic ciliate biomass was comparable between the two lakes. An extreme precipitation event led to a significant increase in bacterial biomass, while simultaneously depressing autotrophic production for several months in the humic lake. Extreme precipitation in the clearwater lake also depressed autotrophic production, but did not give rise to significant increases in bacterial biomass. This quantification of autotrophic,

mixotrophic and heterotrophic components provides vital a first step in understanding how pelagic communities contribute to net ecosystem productivity, and thus how Irish peatland lakes may be affected by projected climate changes.

*Key words:* pelagic plankton, autotrophic, heterotrophic, mixotrophic, humic and clearwater lakes, extreme precipitation.

## Introduction

The relative proportions of carbon derived from allochthonous and autochthonous sources vary widely among lakes, and can change substantially over the annual cycle at an individual site. These variations have consequences for both the composition of the lake biota and for ecosystem functioning. In highly productive lakes, primary production from phytoplankton (autochthonous carbon) is generally the dominant carbon source, while in many oligotrophic lakes, particularly in humic catchments with organic peat soils, allochthonous carbon can be more important (Jansson et al. 2000, Kritzberg et al. 2004, Guillemette et al. 2013). When levels of humic compounds are high, bacterioplankton are likely to be the main pathway by which carbon enters the base of the pelagic food web (Del Giorgio et al, 1997, Attermeyer et al. 2013). These bacteria utilise and mobilise energy from humic substances (Jones 1992, Laybourn-Parry et al. 1994), and then pass it through the food web via phagotropic flagellates and ciliates to zooplankton and hence to fish. A significant proportion of fish biomass can be supported by this terrestrial energy source, particularly in forested catchments (Tanentzap et al. 2013) where leaf litter is a significant source of energy (Carpenter et al. 1998). There is also now strong evidence that many water bodies, even clearwater lakes, may be net heterotrophic aquatic systems, with respiration exceeding gross primary production over the annual cycle (Rubbo et al. 2006, Bass et al. 2010, Ojala et al. 2011, Obrador et al., 2014).

Humic lakes are very common in the northern temperate climate zone, where extensive peat soils are common (Dillon and Molot 1997, Kortelainen 1999, Ojala et al. 2011). These lakes play a significant role in regional and global carbon cycles (Cole et al., 2007; Tranvik et al. 2009). While temperate zones are generally net sinks of CO<sub>2</sub> from the atmosphere (Apps et al. 1993), the lakes within these zones may be significant sources of CO<sub>2</sub> to the atmosphere (Bass et al. 2010). Even minor changes in the quality and quantity of

the humic compounds which are exported to these lakes can have considerable significance for carbon cycling and have substantial ecological consequences in aquatic systems (Cole et al. 2000, Porcal et al. 2009), including shifts in the structure and function of the microbial components of food webs (Jones 1992, Kostrzewska-Szlakowksa and Jasser 2011, Sucker and Krause 2010).

The biota in humic lakes includes not just autotrophs and heterotrophic bacteria, but also mixotrophs. Mixotrophic phytoflagellates can obtain energy via both auto- and heterotrophy and thus function on more than one trophic level by acting as producers and consumers of organic carbon (Jansson et al. 2000, Jones 1994, 2000). Mixotrophy is evident during reduced light conditions (Jones 1997) and enables mobile phytoflagellates to outcompete purely autotrophic species in nutrient limited conditions (Gervais 1997, Bergström et al. 2001). It is a particularly advantageous strategy in humic lakes, where access to nutrients can be restricted due to competition with heterotrophic bacteria (Riemann et al. 1995, Jansson et al. 2001). In these lakes, autotrophic production can also be restricted either by poor light availability (Eloranta 1978, Arvola et al. 1999a) or by inorganic nutrient limitation (Meili 1992) or by both these factors (Drakare et al. 2002, 2003). Humic lakes, therefore, provide conditions favourable for the growth of a diverse pelagic community of mixotrophic phytoflagellates, heterotrophic nanoflagellates, ciliates and bacteria (Isaksson et al. 1999, Jansson et al. 2000, Drakare et al. 2002).

It is widely acknowledged that humic ecosystems are especially sensitive to climate change (Tarnocai 2006, Ise et al. 2008), both to gradual warming and any increased incidence of extreme precipitation. The positive feedback between higher atmospheric CO<sub>2</sub> levels, rising temperatures (IPCC 2013) and then further loss of soil carbon through increased decomposition has major implications for lakes. Higher DOC export as a result of climate warming (e.g. Naden et al. 2010) is likely to further enhance the role of heterotrophs in lakes (De Senerpoint Domis et al. 2013), leading to even greater carbon emissions to the

atmosphere. In addition, while changes in DOC export have received considerable attention (e.g. Jennings et al. 2010, Miller and McKnight 2010), the drivers of particulate organic carbon (POC) export are also influenced at broad spatial scales by climate (Ryder et al. 2014), and therefore also likely to change in the future.

The severity and frequency of extreme precipitation events is projected to increase in the coming decades (Beniston et al. 2007), a change that could affect the normal seasonal cycle of pelagic food webs. Some recent studies have shown that responses can be varied, depending on the magnitude and timing of the event (Jones et al. 2007, Jennings et al. 2012). Increases in nutrient concentrations associated with high flows or mixing events can have a positive impact on both primary and heterotrophic production (Bergström and Jansson 2000, Drakare et al. 2002). High flow events in humic catchments can result in a pulse of coloured water and reduced light availability in the lake that can persist for months or years (Jennings et al. 2012). Intense precipitation may also result in physical disturbance of the water column (Yount 1961, Jones et al. 2007) and can be accompanied by increased export of dissolved and particulate substances to the lake (Weyhenmeyer et al. 2004, Arvola et al. 2006). There is, however, little information available on the effects of such extreme events on the bacterioplankton in temperate humic lakes.

The IPCC (Intergovernmental Panel for Climate Change) recently recommended that emissions from wetlands (including peatlands and lakes) be incorporated into national estimates of greenhouse gas emissions (IPCC 2013). To ensure successful implementation, a greater understanding of the role of heterotrophic and mixotrophic components in the carbon cycle is required. While the literature describing the plankton of humic lakes is immense, there are regions that have never, or rarely, been studied. It is estimated, for example, that there are in excess of 12,000 lakes in Ireland (Irvine et al. 2007). Approximately one-fifth (18.5%) of the country has peaty soils (Montanarella et al. 2006), and therefore a significant proportion of these lakes are humic. Yet despite this, there is just

one summary paper on plankton biomass functional groups (de Eyto and Irvine 2005 ) and no published material on the seasonal dynamics of pelagic food webs in these lakes.

The purpose of this study was to quantify and describe the components of the pelagic auto- (phytoplankton), mixo- (phytoflagellates) and heterotrophic (bacteria and ciliates) fractions of the pelagic communities over an annual cycle in two temperate lakes, both of which are located in peatland catchments, but which differ greatly in their water colour levels, with one being classified as a humic lake and one as a clearwater lake. The study also documents the effects of extreme precipitation events on the pelagic community in both lakes.

## Methods

The two study lakes, Feeagh and Guitane, are of a relatively similar size, and both have catchments that are dominated by peatland, but they differ in their catchment area to lake area ratio (Fig. 1). Feeagh (53°94'05"N, 9°57'59"W) is located within the Burrishoole catchment (County Mayo) on the northwestern coast of Ireland and is part of the Global Lake Ecological Observatory Network (GLEON, [www.gleon.org](http://www.gleon.org)). It is also an internationally important index site for the monitoring of diadromous fish. The lake has a surface area of 3.95 km<sup>2</sup>, a maximum depth of 46 m, a mean depth 14.5 m, a retention time of 164 days and a lake catchment area of 89.5 km<sup>2</sup>, giving it a catchment area:lake area ratio of 22.7. Guitane (52°00'48"N, 9°41'83"W) is located within the Leane catchment (County Kerry) in the southwest of Ireland and is the primary water source for approximately 59,000 people (EIS 2009). It has a lake area of 2.46 km<sup>2</sup>, with slightly deeper maximum (56 m) and mean depths (18.7 m), a retention time of 167 days but has a lake catchment area of only 18.69 km<sup>2</sup>, and therefore a catchment area:lake area ratio of 7.6. The land cover in the Feeagh catchment includes 58% peatland, 22% coniferous forestry and 5% water bodies as well as some natural

grassland, transitional woodland and agricultural land (CORINE 2006). The Guitane catchment includes 69% peatland, 14% water bodies and 7% pasture together with limited broadleaved forest and sparsely vegetated areas. There is no coniferous forestry in the Guitane catchment (CORINE 2006). Rainfall and air temperature data were available from meteorological stations within or close to the catchments. The average annual precipitation at the meteorological station in the Burrishoole catchment (1970 to 2012) was 1586 mm year<sup>-1</sup>, while that for the Muckcross meteorological station 15 km from Guitane (1990-2009) was 1833 mm year<sup>-1</sup>.

The two lakes were monitored from April 2009 to May 2010. An Automatic Water Quality Monitoring System (AWQMS) on Feeagh enabled the measurement of vertical temperature profiles using platinum resistance thermometers (PRTs: PT100 1/10DIN four wire sensor, Lab facility) and high frequency measurements of turbidity using a nephelometer (Chelsea Scientific Minitracka mk II). Daylight hours were quantified using the NOAA ESRL calculator (<http://www.esrl.noaa.gov/gmd/grad/solcalc/sunrise.html>). Vertical water temperature profiles from Guitane were collected on a monthly basis. Thermocline depth was calculated using Lake analyzer web (<http://lakeanalyzer.gleon.org>) (Read et al., 2011). Secchi depth was recorded on all sampling occasions at both sites.

Vertically integrated open water samples were collected every month from the deepest point of each lake. Poor weather in November necessitated collection of an outflow sample (Finow river) from Guitane. Water samples were collected using a 2.5 cm diameter tube sampler with two lengths: 1.5 m for Feeagh and 5 m for Guitane. The different lengths accommodated the annual average Secchi depths of the last five years (2003-2008; n=60), or the mean depth of the euphotic zone (Håkanson and Peters 1995, Arvola et al. 1999b). A 250 mL sub-sample for phytoplankton and ciliate analysis was fixed with 1.5 mL of Lugol's iodine solution (Merck with a composition of I<sub>2</sub> = 3.2 g L<sup>-1</sup> and KI = 6.8 g L<sup>-1</sup>) (European Union, 2009). Samples for pico- and bacterioplankton analysis were fixed with pre-filtered (0.2 µm pore

size, Whatman GTTPO2500) 20% formaldehyde buffered with sodium cacodylate 0.1 M to final concentrations of 1% and 4%, respectively (Hayat, 1981) and stored in sterilized amber glass bottles (Callieri and Stockner 2002). The use of 20% formaldehyde is considered less stressful for cells (Callieri and Stockner 2002). The samples were kept refrigerated in the dark and were processed as soon as possible after sampling to avoid loss of cell numbers (Turley and Hughes 1992) and to decrease problems with bleaching of autofluorescent pigments (Ollrik et al. 1998, Callieri and Stockner 2002). Alkalinity, conductivity, pH, total phosphorus (TP), dissolved molybdate reactive phosphorus (DMRP), total nitrogen (TN), nitrate (as nitrogen  $\text{NO}_3\text{-N}$ ) and chlorophyll *a* (Chl-*a*) were analysed using standard methods (Eisenreich et al. 1975, Korolef 1983, Standing Committee of Analysts 1983, Davison 1990, Clesceri et al. 1999). Filtered samples (Whatman glass microfiber filter GF/F 0.45  $\mu\text{m}$  pore size) were measured for colour using a Hach DR 5000 UV Vis Spectrophotometer in platinum cobalt units ( $\text{mg L}^{-1}$  PtCo) while DOC (in  $\text{mg L}^{-1}$ ) was measured using a TOC Elementar (model Vario TOC cube) analyzer (Clesceri et al. 1999).

Phytoplankton and ciliate cell enumeration was determined using the Utermöhl (1958) sedimentation technique. The characterization of auto- and mixotrophic species of phytoplankton was carried out according to Jansson et al. (1996) with modifications according to Isaksson et al. (1999). Bacterio- and picoplankton samples were processed where possible within one week in the laboratory following the method described by Sherr et al. (1993) and MacIsaac and Stockner (1993). A wetted white polycarbonate filter (Millipore, Ireland, type HAWPO2500) was placed on the filtering device to support the membrane filter in order to facilitate even distribution of the sample. Subsamples of 1 and 5 mL were filtered onto 0.2  $\mu\text{m}$  pore-sized black isopore membrane filters (Millipore, Ireland, type GTBP 2500) and, in semi-darkness, 0.1 and 0.5 mL of  $0.1 \mu\text{g mL}^{-1}$  4'6'-diamidino-2-phenylindole (DAPI) were added. The whole sample was drawn through the filter with a vacuum pump under low pressure (5-10 kPa) (Kuuppo-Leinikki and Kuosa 1989, MacIsaac 8



and Stockner, 1993). For the picoplankton, two 5 mL subsamples underwent the same procedure without the addition of DAPI. The filters were dried after removal from the holder and mounted on glass slides directly on a small drop of 50% glycerol-water solution (Callieri and Stockner 2002a) with a cover slip. The slides were stored at  $-20^{\circ}\text{C}$  to minimize bleaching of the autofluorescent pigments (Maclsaac and Stockner 1993).

Bacteria and picoplankton were counted under an epifluorescence microscope (ZEISS Axioplan). Bacteria were counted using a UV filter (G365, FT395, LP420), while picoplankton were examined using filters for blue (BP450-490, FT510, LP520) and green light excitation (LP510-KP560, FT580, LP590). The fluorescent cells observed on the filters were enumerated in random fields at the highest magnification (x1250). At least 400 cells were counted with an upper limit set at 30 fields to obtain a precision of 10% (Lund *et al.*, 1958). The heterotrophic bacteria appeared bright blue in colour against a dark background. Conversion factors and other methodological specifications followed Straškrabová *et al.* (1999). It must be noted that the picoplankton samples were not always processed immediately after field sampling, and for this reason the picoplankton counts are probably underestimated. Heterotrophic nanoflagellates were not enumerated for this study.

Phytoplankton and ciliate biomass were calculated by multiplying the number of cells of a given species counted in a sample by its average cell volume (calculated using digital photographs and the direct measurement of the linear dimensions) (Potapova and Snoeijs 1997, Hillebrand *et al.* 1999). The biovolume of the dominant species were calculated according to 20 different geometric shapes and respective equations taken from the literature (e.g. Hillebrand *et al.* 1999, Sun and Liu 2003, Vadrucci *et al.* 2007). Digital images (Image Pro Plus Version 4.5.1) of pico- and bacterioplankton were used to measure volume of at least 100 cells according to the algorithms given in Massana *et al.* (1997). Multivariate analysis of the biological data was carried out using the package VEGAN (Oksanen *et al.* 2013) in R (version 3.0.2) (R Core team 2013). Ordination of samples was

carried out using nMDS (multidimensional scaling) whereby the placement of samples reflects the similarity of their biological communities. Biological data were square root transformed to down-weight the effect of very abundant groups. Formal tests for differences between assemblages were carried out using ANOSIM (analysis of similarity) which tests the Null hypothesis that there are no assemblage differences between groups of samples.

## **Results**

Annual rainfall totals of 1,657 mm year<sup>-1</sup> in Burrishoole (Feeagh) and 2,023 mm year<sup>-1</sup> in Leane (Guitane) were recorded between May 2009 and April 2010. The wettest months in Burrishoole were July (243.8 mm month<sup>-1</sup>), August (254.7 mm month<sup>-1</sup>) and November (322.9 mm month<sup>-1</sup>) and the coldest temperature of -1°C was registered at the end of December 2009. An extreme rainfall event occurred on the 2<sup>nd</sup> July 2009 on the east side of the Burrishoole catchment, when 52 mm of rain was recorded over a period of two hours. It is estimated that this type of extreme rainfall event has an expected return period in excess of 250 years in Ireland (Fitzgerald 2007). At Guitane, the wettest months were July (218.3 mm month<sup>-1</sup>), October (223.3 mm month<sup>-1</sup>) and November (485.1 mm month<sup>-1</sup>) 2009, while the coldest temperature of -6.6 °C was recorded at the beginning of January 2010. A deep Atlantic depression brought very wet and windy conditions to the southwest of Ireland in November and persistent and very heavy rain, the highest on record (Walsh 2010). This led to unprecedented levels of flooding in many parts of the catchment in that month. The variation in daylight hours in Feeagh ranged from a maximum of 17.2 hours in July 2009 to a minimum of 7.5 hours in January 2010. Daylight hours in Guitane ranged between 16.7 in July 2009 and 7.9 hours in January 2010.

The water temperatures in Guitane were generally slightly higher than those in Feeagh, although the two study lakes had a relatively similar pattern in their temperature profiles over the study period (Fig. 2), with both lakes exhibiting thermal stratification in summer months. In Feeagh, the maximum temperature differential between the epilimnion and hypolimnion was recorded in July at 5.1 °C, while that of Guitane was 8.9 °C in the same month. This difference between the sites reflected, in part, colder deep water temperatures in Guitane during summer. The water column of both lakes mixed in November 2009, remaining isothermal over the winter and early spring. The two lakes had similar pH and alkalinity levels (mean pH 6.9 for both lakes and mean alkalinity of 5 and 5.5 mg L<sup>-1</sup> CaCO<sub>3</sub> for Feeagh and Guitane respectively), while Feeagh had generally higher conductivity, DOC, colour and nutrient levels and Guitane had higher concentrations of chlorophyll *a* (Table 1). Water colour was on average four times higher in Feeagh than in Guitane (84 and 21 mg L<sup>-1</sup> PtCo respectively) and reached a maximum in August 2009 in Feeagh, and in November 2009 in Guitane (Fig. 3). DOC concentration ranged from 6.2 to 11.4 mg L<sup>-1</sup> in Feeagh and from 1.5 to 6.4 mg L<sup>-1</sup> in Guitane. Secchi depth in Feeagh ranged between 1.2 to 2.1 m, while that of Guitane ranged between 4.4 and 5.7 m, reflecting the difference in water colour at the two sites. Chlorophyll *a* values were low (average of 1.1 and 2.1 mg L<sup>-1</sup> in Feeagh and Guitane respectively), but indicated very similar seasonal patterns of phytoplankton dynamics in the two lakes, with highest values in mid summer, dropping to winter lows in January and February (Fig. 3). Total phosphorus was generally low in both lakes, with annual averages falling into the oligotrophic class (<10 µg L<sup>-1</sup>) as defined by the OECD (1982). The exception to this was the sample taken in November from Guitane, which was a spike of 16 µg L<sup>-1</sup>. This sample was taken from the lake outflow rather than mid lake due to poor weather conditions, although it should be noted that none of the other physical or chemical variables appear to be affected. A proxy measurement for turbidity (nephelometer readings in millivolts) (Fig. 4) increased after the July flood event in Feeagh. It took three months for

these readings to return to pre-flood values. No turbidity measurements were available for Guitane.

The total biomass of pelagic organisms quantified in this study differed significantly between the two lakes (t-test,  $t = -2.4901$ ,  $df = 22$ ,  $p\text{-value} = 0.021$ ) (Fig. 5). The average pelagic biomass of Feeagh was  $366 \text{ mm}^3 \text{ m}^{-3}$  over the annual cycle, while that of Guitane was  $581 \text{ mm}^3 \text{ m}^{-3}$ . In general, the largest portion of the pelagic biomass in Feeagh was made up of bacteria (average of  $153 \text{ mm}^3 \text{ m}^{-3}$ ), followed by autotrophic phytoplankton (average  $134 \text{ mm}^3 \text{ m}^{-3}$ ). In Guitane, autotrophic phytoplankton biomass was higher (average of  $355 \text{ mm}^3 \text{ m}^{-3}$ ) (Fig. 5) than that of bacteria ( $103.4 \text{ mm}^3 \text{ m}^{-3}$ ). The ratio of phytoplankton (P: including mixotrophic species) to bacteria (B) biomass for each sampling occasion summarises this difference in the sites, with the annual PB ratio in Feeagh averaging 1.17 (i.e. roughly similar biomass of phytoplankton and bacteria when mixotrophic species are included). In contrast, the PB ratio of Guitane averaged 4.27 (i.e. four times more phytoplankton than bacteria over the annual cycle) (Fig. 5). When potentially mixotrophic species (*Rhodomonas* sp., *Cryptomonas* sp., *Chrysochromulina parva* (Lackey), *Dinobryon* sp., *Ochromonas tuberculata* (D.J.Hibberd), *Gymnodinium uberrimum* (G.J.Allman) Kofoid & Swezy and *Gymnodinium triceratium* (Skuja) are considered independently of the rest of the phytoplankton, it is apparent that Feeagh supports a lower biomass of potential mixotrophs than Guitane (annual average of  $30.9$  and  $82.7 \text{ mm}^3 \text{ m}^{-3}$  respectively). However, the relative contribution of mixotrophic species in the overall phytoplankton biomass was similar in both lakes (annual average of 21% in Feeagh and 25% in Guitane). The biomass of phagotrophic ciliates were roughly similar in the two lakes, averaging  $48 \text{ mm}^3 \text{ m}^{-3}$  in Feeagh over 12 months, and  $37 \text{ mm}^3 \text{ m}^{-3}$  in Guitane. The average annual biomass of autotrophic phytoplankton, bacteria, potentially mixotrophic phytoplankton and ciliates represented 36%, 43%, 6% and 15% respectively of the pelagic biomass in Feeagh. In Guitane, the equivalent percentages were 57%, 20%, 15% and 8%.

The biomass of the constituent parts of the pelagic biomass also varied significantly between lakes (ANOSIM statistic  $R = 0.46$ ,  $p < 0.001$ ), resulting in clear separation of Feeagh samples from Guitane samples when plotted using nMDS (Fig. 6). The high relative biomass of autotrophic Chrysophyta (*Mallomonas akrokomos* and *Mallomonas caudate*), bacteria and picoplankton distinguished the pelagic community of Feeagh. In contrast, the pelagic plankton of Guitane had relatively high biomass of the potentially mixotrophic Dinophyte *Ceratium hirudinella*, along with substantial biomass of cyanophytes and chlorophytes. Significant environmental vectors overlaid on the nMDS plot ( $p < 0.05$ , Fig. 6) indicate that the split between the pelagic foodwebs of the two lakes (i.e. top left to bottom right) can be best explained by the humic characteristics of the water (as indicated by colour and Secchi depth). The perpendicular vectors of pH, light and temperature indicate the difference between summer samples (plotted towards the bottom left of Fig. 6) and winter samples (plotted towards the top right of Fig. 6) in both lakes. The presence of the autotrophic dinophyte *Ceratium hirudinella* (O.F.Müller) characterised the samples taken in July 2009 from both lakes.

In Feeagh, the biomass of autotrophic phytoplankton (including picoplankton) was highest in the first two months of sampling during spring 2009 (Fig. 5). This biomass decreased substantially after the flood event on the 2<sup>nd</sup> July (the July sample was taken three weeks after the flood event), recovered slightly over the summer and then dropped to a winter low between November and February 2009/2010. The potentially mixotrophic phytoplankton biomass followed a relatively similar pattern, being highest in May, and then decreasing in the weeks after the flood. A small increase in biomass was noted in August, before declining for the rest of the year. In contrast, both phagotrophic ciliate biomass and heterotrophic bacterial biomass increased in the month after the flood. Ciliate biomass also exhibited a peak in January 2010.. Diatoms (predominantly *Asterionella formosa* Hassall and *Aulacoseira alpigena* (Grunow) Krammer) were common in all months. Samples from June to

September were characterised by the presence and relatively high abundance of the autotrophic dinophyte *Ceratium hirundinella*. A bloom of the cyanophyte *Woronichinia naegeliana* (Unger) Elenkin was noted in August. The Cryptophyta *Rhodomonas* sp. and *Cryptomonas* sp. were also relatively frequent over the summer months.

Despite the seemingly obvious effect of the flood event on the biomass of various groups (as shown in Fig. 5), neither rainfall nor turbidity was significantly correlated with the ordination in Fig. 6. Instead, the pelagic communities appear to be most affected by seasonal changes in temperature, light and pH, which enabled diverse summer populations of cyanophytes, mixotrophic cryptophytes, dinophytes, and chrysophytes to develop. In contrast, when the lake was fully mixed in winter, the winter samples are dominated by bacteria, ciliates and picoplankton.

The autotrophic phytoplankton bloom in Guitane lasted longer than that of Feeagh, with high biomass being recorded right through until September 2009 (Fig. 5). Chlorophyte biomass was highest between June and September and contained a diverse mix of species including *Cosmarium tinctum* Ralfs, *Crucigenia tetrapedia* (Kirchner) Kuntze, *Monoraphidium minutum* (Nägeli) Komárková-Legnerová, *Sphaerocystis Schroeteri* Chodat and *Spondylosium planum* (Wolle) West & G.S.West. Late spring and early summer samples were characterised by relatively high biomass of autotrophic Chrysophyta and Dinophyta. While cell densities of colonial picocyanobacteria (*Aphanocapsa* sp., *Aphanothece* sp., *Merismopedia* sp.) were very high in the summer months, their relative biomass was low. The extensive precipitation of November had no noticeable effect on the species composition of the pelagic community, however, a notable bloom of *Tabellaria flocculosa* var. *asterionelloides* (Grunow) Knudson ( $198 \text{ mm}^3 \text{ m}^{-3}$ ) was recorded in December. In general, diatom biomass remained consistently important throughout the annual cycle, as indicated by its placement in the centre of the ordination plot.

In both lakes, extreme precipitation events led to a decrease in phytoplankton biomass as indicated by chlorophyll *a* values (Fig. 7). When viewed in comparison to long term monthly average chlorophyll *a* values (2007-2014 for Feeagh, 1999-2008 for Guitane), the chlorophyll *a* values after the precipitation events in both lakes were considerably lower than normal for the relevant time of year (August to January in Feeagh and December to March in Guitane) indicating depressed autotrophic production. While bacterial biomass increased in Feeagh after the flood from 218 to 324 mm<sup>3</sup> m<sup>-3</sup>, a similar pattern was not observed in Guitane, with bacterial biomass remaining low (circa 95 mm<sup>3</sup> m<sup>-3</sup>) in the months following heavy rainfall in November 2009 (Fig. 5).

## Discussion

Comparisons of water colour and nutrient concentrations from both lakes confirm the contrasting characteristics of Feeagh and Guitane, despite their similar peatland dominated catchments, and their similar Irish EPA preliminary Water Framework Directive (WFD) typology classification (Lake Type 4: deep (average > 4m and maximum depth > 12 m), surface area > 50 ha and low alkalinity (< 20 mg L<sup>-1</sup> CaCO<sub>3</sub>)) (Free et al. 2002). Catchment percentage peat cover is sometimes cited as a good predictor for DOC concentrations in catchment streams (e.g. Hope et al. 1994, Aitkenhead et al. 1999). However, if the most recently formulated classification scheme for the WFD (Poikane 2009) is applied, Feeagh and Guitane actually fall into separate lake types within the Northern Geographical Intercalibration Group: humic (water colour 30-90 mg PtCo L<sup>-1</sup>) and clearwater (water colour < 30 mg PtCo L<sup>-1</sup>), respectively. Both lakes experienced seasonal variation in water colour during the sampling period, with highest levels in autumn and early winter, but in contrasting ranges. While nutrient concentrations were higher in Feeagh, nutrient

availability was probably restricted due to its high content of humic substances. Nürnberg and Shaw (1999) described higher geometric means of summer surface water TP and TN concentration in coloured lakes than in clearwater lakes. In coloured lakes, these key nutrients are largely bound with fulvic and humic compounds (Tipping 1981, Jansson et al. 2001) and therefore their dissolved concentrations and bioavailability are limited (Perdue 1998, Shaw et al. 2000). Moreover, the NP-ratios of both lakes were greater than 17 and confirm that both lakes were phosphorus limited (Smith 1982, Nürnberg and Shaw 1999).

The comparison of the algal communities showed that autotrophy was more important in the pelagic foodweb of Guitane than in that of Feeagh. In contrast, heterotrophy likely supported a higher portion of the energy transfer to higher trophic levels in Feeagh, given the absence of any significant biomass of autotrophic phytoplankton. The annual phytoplankton biomass in Guitane was equally distributed among several algal groups (Chlorophyta, Cyanophyta, Cryptophyta and Bacillariophyta) and their increase in summer and early autumn coincided with increased surface water temperatures and light availability. In contrast, heterotrophic bacteria were the most abundant organisms in Feeagh. Generally, light penetration is limited in lakes with highly coloured water, and can potentially restrict primary production and biomass (Jones 1992, Carpenter et al. 1998, Jansson et al. 2000), and the results presented here support this pattern. Water transparency in Guitane was higher compared to Feeagh, thus leading to a much larger habitat area for successful autotrophic phytoplankton growth. The ordination analysis confirmed that water colour (and its correlated variables DOC and Secchi depth) was the most significant environmental variable associated with the difference in the pelagic communities in both lakes.

The phytoplankton : bacteria biomass ratio is generally low in humic lakes (Tranvik 1989, Bergström et al. 2003), and the bacterial production may be several times higher than the planktonic primary production (Tranvik 1989). This was evident in the study lakes with



an annual mean ratio of 1.7 in Feeagh and 4.27 in Guitane. Moreover, the purely autotrophic biomass (phytoplankton not including mixotrophs) was generally lower over the annual cycle in Feeagh (except spring when diatoms increased) compared to the bacterial biomass. In contrast, phytoplankton dominated the pelagic biomass in Guitane. These results conform to several studies in humic lakes, which have documented that biomass and productivity of the heterotrophic bacteria can equal or be much greater than that of autotrophic communities in the euphotic zone (Jansson et al. 1996, Nürnberg and Shaw 1999, Jansson et al. 2001, Staehr et al. 2011). This has implications for the role that individual lakes play in carbon cycling within a catchment. Because of the predominance of bacterial biomass in the pelagic zone, it is likely that Feeagh is a net heterotrophic lake over the annual cycle, and thus acts as a carbon source to the atmosphere. Recent metabolism studies (Solomon et al. 2013) of Feeagh have indicated that this may be the case, with respiration exceeding gross primary production in the epilimnion in 2006, resulting in negative NEP (net ecosystem productivity) over the whole year. Guitane is also situated in a predominantly peat catchment, and it was expected that heterotrophic bacteria would likewise play an important role in processing allochthonous carbon. The data presented here indicate that gross primary production in the epilimnion is likely to be higher than respiration, given the relatively higher biomass of autotrophs. However, if the whole lake is examined, significant heterotrophic productivity may be evident as considerable respiration may occur below the euphotic zone (Obrador et al. 2014).

The quantification of autotrophs and heterotrophs, as presented in this study of two contrasting lakes within peatland catchments, is a first step in understanding the role that these lakes play in processing terrestrial carbon stores. Future avenues for exploration include the calculation of whole lake metabolism for a range of temperate peatland catchments in this geographic area, to quantify the contribution of pelagic organisms to NEP.

Given the reported (Worrall and Burt 2007, Bates et al. 2008) and projected rises in DOC (Naden et al. 2010), this work would be particularly timely.

With the exception of the month after the flood for Feeagh, bacterial biomass in the two lakes was of a similar order of magnitude (Fig. 5). It is surprising, therefore, that the biomass of potentially mixotrophic species was almost twice as high in Guitane than in Feeagh. Although primary production by autotrophs is probably the main route of energy to higher trophic levels in Guitane, there may be comparable quantities of heterotrophy in the two lakes (given the similar quantities of bacterial biomass). Thus, the cumulative amount of energy available to zooplankton and fish from these two sources was higher in Guitane than in Feeagh. Bacterial mineralization of terrestrial carbon is known to be significant in clearwater lakes (Karlson et al. 2002), but is probably sometimes overlooked when there is obvious primary production by phytoplankton. It is also worth noting that even though assimilation of allochthonous carbon into the pelagic foodweb via heterotrophs can be substantial, the nutritional quality of this resource is low (Kelly et al. 2014). In addition, the degree of heterotrophy in the potentially mixotrophic species may have varied between lakes and over time. Light attenuation in Feeagh makes it more likely that the mixotrophic species outcompete other phytoplankton species (Morgan and Kalff 1979) by utilising the bacterial biomass as an energy source. In Guitane, with a euphotic zone of several metres depth, the mixotrophic species also have the option of boosting their metabolism via autotrophy, leading to larger biomass accumulations despite the similar sized bacterial resource.

Extreme precipitation events are known to have strong impacts on aquatic ecosystems (Parmesan et al. 2000). Flash floods increase the inflow of allochthonous inorganic suspended solids from the catchment. Jennings et al. (2010) described the effects of pulses in dissolved organic matter from rivers into downstream lakes, which included prolonged decreases in lake water clarity. Data collected immediately after large flood events at the Swedish Lake Östräsket

showed that such increases in the supply of labile organic substrates from the catchment stimulated bacterial production in the lake (Bergström and Jansson 2000, Drakare et al. 2002). Berggren et al. (2010) have shown that such responses can be the result of assimilation of low-molecular-weight DOM from terrestrial sources in the bacterial foodweb. The bacterial biomass in Feeagh followed a very similar pattern, reaching a peak in the month after the flood event in July 2009, and then decreasing steadily over the following 9 months. The physicochemical data indicated that the main input to Feeagh with the floodwaters was particulate matter, as the turbidity sensor showed an immediate spike, while DOC and water colour showed no significant rise between June and July. A recent study by Guillemette et al. (2013) indicated that bacterioplankton can utilise both allochthonous and autochthonous sources of carbon, albeit over different time scales, so it is likely that the increased input of carbon after the flood (especially in particulate form) was able to stimulate the growth of bacterial populations. The same stimulating effect of humic substances on growth and abundance of bacteria has been documented in many Scandanavian lakes (e.g. Tranvik 1988, Weyhenmeyer et al. 2004).

Although the rainfall in the Guitane catchment in November 2009 was the highest on record, the biomass of bacteria, ciliates and mixotrophic phytoplankton remained constant over the subsequent winter months (Fig. 3). A small increase in phytoplankton biomass was evident between November and December, which was mainly due to *Tabellaria flocculosa* var. *asterionelloides*. Winter blooms of large diatoms are well documented (Twiss et al. 2012, Kerfoot et al. 2008), but without a long term record, it is impossible to say whether this increase was related to higher precipitation levels. Chlorophyll *a* was low relative to longterm records in the months post-flood at both sites, even though the events occurred at different times in the growing season. Although data were lacking for the physical and chemical effects of the floods in Guitane, the data from Feeagh suggested that the decrease in chlorophyll *a* may have been due to increased turbidity. However, it is also of note that Jennings et al. (2000) reported lower winter chlorophyll *a* in Leane, downstream of Guitane,

in winters with a higher North Atlantic Oscillation index, which are characterised by increased rainfall. Washout of smaller phytoplankton species has also been reported following high rainfall from other lakes (Arvola et al. 1996). Our results suggest that in both lakes, the extreme precipitation events of 2009 can be considered significant disturbances. The Intermediate Disturbance Hypothesis (Connell 1978) predicts a decrease in biomass following a significant disturbance, whereas the normal response of phytoplankton to predictable seasonal rainfall is more likely to be an increase in biomass (Paidere et al. 2007, Mihaljević et al. 2009). The results presented here indicate that one of the likely outcomes of increases in extreme precipitation events in temperate lakes will be an overall decrease in gross primary productivity as suggested by some future climate simulations (e.g. Jones et al. 2011). The results from Feeagh also indicate that some of this productivity loss may be compensated for by increased bacterial heterotrophy. However, the low nutritional quality of this heterotrophic mobilisation of terrestrial carbon means that overall energy transfer to zooplankton and fish is likely to be lower (Kelly et al. 2014).

## **Conclusions**

The pelagic planktonic communities of Guitane and Feeagh conform to expectations derived from previous studies of clearwater and humic lakes. Despite their common situation in peatland-dominated catchments, the results indicate that a more significant proportion of the pelagic plankton in Feeagh, the more humic lake, was heterotrophic. Light restrictions caused by humic compounds probably limited the role of autotrophs, while the supply of DOC and POC supported significant biomass of heterotrophic bacteria. Bacterial biomass in the humic lake was especially high in the month following a flash flood in the catchment, which brought with it large amounts of particulate matter. In both lakes, chlorophyll *a* levels were depressed in the months following extreme precipitation events. The results presented

here provide valuable empirical information, quantifying the temporal variability in the pelagic communities controlling lake metabolism, and in particular their response to extreme precipitation events. Given the high proportion of humic lakes in western and northern Europe, and the projected increasing levels of aquatic DOC with climate change, it is probable that carbon emissions from these lakes are likely to increase in the future.

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## **References**

- Aitkenhead JA, Hope D, Billett M. 1999. The relationship between dissolved organic carbon in stream water and soil organic carbon pools at different spatial scales. *Hydrological Processes*. 13:1289-1302.
- Arvola L, Kankaala P, Tulonen T, Ojala A, 1996. Effects of phosphorus and allochthonous humic matter enrichment on the metabolic processes and community structure of plankton in a boreal lake. *Canadian Journal of Fisheries and Aquatic Sciences*, 53:1646–1662.
- Arvola L, Eloranta P, Järvinen M, Keskitalo J, Holopainen AL, 1999a. Food webs of humic waters, p. 137-160. In J. Keskitalo and P. Leiden (eds.), *Limnology of Humic Waters*. Leiden, Netherlands: Backhuys Publishers.

- Arvola L, Eloranta P, Järvinen M, Keskitalo J, Holopainen AL, 1999b. Phytoplankton, p. 137-171. In J.P.E. Keskitalo (ed.), *Limnology of Humic Waters*. Leiden, The Netherlands. Backhuys Publishers.
- Attermeyer K, Premke K, Hornick T, Hilt S, Grossart H P, 2013. Ecosystem-level studies of terrestrial carbon reveal contrasting bacterial metabolism in different aquatic habitats. *Ecology*, 94(12): 2754-2766.
- Bates BC, Kundzewicz ZW, Wu S, Palutikof JP, 2008. *Climate change and water*. Geneva: Technical paper of the Intergovernmental Panel on Climate change. IPCC Secretariat.
- Berggren M, Laudon H, Mahsa Haei M, Ström L, Jansson M, 2010. Efficient aquatic bacterial metabolism of dissolved low-molecular-weight compounds from terrestrial sources. *The ISME journal* 4(3): 408-416.
- Bergström AK, Jansson M, 2000. Bacterioplankton production in humic Lake Östräsket in relation to input of allochthonous organic carbon. *Microbial Ecol.* 39:101-115.
- Bergström AK, Jansson M, Blomqvist P, Drakare S, 2001. The influence of water colour and effective light climate on mixotrophic phytoflagellates in three small dystrophic Swedish lakes. *Verh. Internat. Verein Limnol.* 27:1861-1865.
- Bergström AK, Jansson M, Drakare S, Blomqvist P, 2003. Occurrence of mixotrophic flagellates in relation to bacterioplankton production, light regime and availability of inorganic nutrients in unproductive lakes with differing humic contents. *Freshwater Biol.* 48:868-877.
- Callieri C, Stockner JG, 2002. Freshwater autotrophic picoplankton: a review. *Journal of Limnology.* 61: 1-14.
- Carpenter SR, Cole JJ, Kitchell JF, Pace ML, 1998. Impact of dissolved organic carbon, phosphorus and grazing on phytoplankton biomass and production in experimental lakes. *Limnol. Oceanogr.* 43:73-80.

- Clesceri LS, Greenberg AE, Trussell RR, 1999. Standard Methods for the Examination of Water and Wastewater, Washington DC.
- Cole JJ, Pace ML, Carpenter SR, Kitchell JF, 2000. Persistence of net heterotrophy in lakes during nutrient addition and food web manipulation. *Limnol. Oceanogr.* 45:1718-1730.
- Cole JJ, Prairie YT, Caraco NF, McDowell WH, Tranvik LJ, Striegl RG, Duarte CM, Kortelainen P, Downing JA, Middelburg JJ, Melack J, 2007. Plumbing the global carbon cycle: integrating inland waters into the terrestrial carbon budget. *Ecosystems.* 10:171-184.
- Connell JH, 1978. Diversity in tropical rain forests and coral reefs. *Science* 199(4335): 1302-1310.
- CORINE. 2006. <http://www.eea.europa.eu/data-and-maps/data/corine-land-cover-2006-raster-1>.
- Davison W, 1990. A practical guide to pH measurement in freshwaters. *Trends in Analytical Chemistry* 9:80-83.
- de Eyto E, Irvine K, 2005. Variation in the biomass of functional groups comprising the open-water plankton of shallow lakes in Ireland. *Biol. Environ.* 105b:53-58.
- Del Giorgio P A, Cole J J, Cimleris A 1997. Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. *Nature* 385(6612) 148-151.
- De Senerpont Domis LN, Elser JJ, Gsell AS, Huszar VLM, Ibelings BW, Jeppensen E, Kosten S, Mooij WM, Roland F, Sommer U, Van Donk E, Winder M, Lürling M 2013. Plankton dynamics under different climatic conditions in space and time. *Freshwater Biol.*, 58:463-482.

- Dillon PJ, Molot LA, 1997. The effect of landscape form on export of dissolved organic carbon, iron, and phosphorus from forested stream catchments. *Water Resour. Res.* 33:2591-2600.
- Drakare S, Blomqvist P, Bergström AK, Jansson M, 2002. Primary production and phytoplankton composition in relation to DOC input and bacterioplankton production in humic lake Östräsket. *Freshwater Biol.* 47:41-52.
- Drakare S, Blomqvist P, Bergström AK, Jansson M, 2003. Relationships between picophytoplankton and environmental variables in lakes along a gradient of water colour and nutrient content. *Freshwater Biol.* 48:729-740.
- EIS (Environmental Impact Services) (2009) Environmental Report on the Proposed Upgrade and Expansion of Water Treatment Plant at Lough Guitane, County Kerry. Environmental Assessment for Kerry County Council, 135pp.
- Eisenreich SJ, Bannerman RT, Armstrong DE, 1975. A simplified phosphorus analysis technique. *Environ Lett.* 9:43-53.
- Eloranta P, 1978. Light penetration in different types of lakes in Central Finland. *Holarctic Ecol.* 1:362-366.
- European Union, 2009. Guidance on the quantitative analysis of phytoplankton in the WISER field campaign lake samples. Brussels, European Committee for Standardization: 15 pp.
- Fitzgerald, D. L. 2007. Estimation of point rainfall frequencies. Met Éireann, Irish Meteorological Service, Technical note 61. Dublin. 53pp.
- Free G, Little R, Tierney D, Donnelly K, Caroni R. 2006. A reference based typology and ecological assessment system for Irish lakes-preliminary investigations. Environmental Protection Agency, Wexford. pp. 266.
- Gervais F, 1997. Light-dependent growth, dark survival and glucose uptake by cryptophytes isolated from a freshwater chemocline. *J. Phycol.* 33:18-25.



- Guillemette F, McCallister SL, del Giorgio PA, 2013. Differentiating the degradation dynamics of algal and terrestrial carbon within complex natural dissolved organic carbon in temperate lakes. *Journal of Geophysical Research*: 118:1-11.
- Håkanson L, Peters RH, 1995. *Predictive Limnology. Methods for Predictive Modelling*. SPB Academic Publishing bv, Amsterdam: 464 pp.
- Hayat MA, 1981. *Fixation for electron microscopy*. Academic Press, New York.
- Hillebrand HD, Kirschtel CD, Pollingher D, Zohary T, 1999. Biovolume calculation for pelagic and benthic microalgae. *J. Phycol.* 35:403-424.
- Hope D, Billet MF, Cresser MS, 1994. A review of the export of carbon in river waters: fluxes and processes. *Environ. Pollut.* 84:301-324.
- Irvine K, Mills P, Donohue I, Fuller J, 2007. Conservation assessments of lake habitats in the Republic of Ireland. Report for the National Parks and Wildlife Service, Department of the Environment, Heritage and Local Government, Ireland: 132 pp.
- Isaksson A, Bergström AK, Blomqvist P, Jansson M, 1999. Bacterial grazing by phagotrophic phytoflagellates in a deep lake in northern Sweden. *J. Plankton Res.* 21:247-268.
- Ise, T, Dunn AL, Wofsy SC, Moorcroft PR. 2008. High sensitivity of peat decomposition to climate change through water-table feedback. *Nat. Geosci.* 1,: 763-766.
- Jansson M, Blomqvist P, Jonsson A, Bergström AK, 1996. Nutrient limitation of bacterioplankton, autotrophic and mixotrophic phytoplankton, and heterotrophic nanoflagellates in lake Öträsket. *Limnol. Oceanogr.* 41:1552-1559.
- Jansson M, Bergström AK, Blomqvist P, Drakare S, 2000. Allochthonous organic carbon and phytoplankton/bacterioplankton production relationships in lakes. *Ecology.* 81:3250-3255.

- Jansson M, Bergström AK, Drakare S, Blomqvist P, 2001. Nutrient limitation of bacterioplankton and phytoplankton in humic lakes in northern Sweden. *Freshwater Biol.* 46:653-666.
- Jennings E, Allott N, McGinnity P, Poole R, Quirke W,, Twomey H, George G, 2000. The North Atlantic Oscillation: effects on freshwater systems in Ireland. *Biol. Environ.* 100B: 149-157.
- Jennings E, Allott N, Arvola L, Jarvinen M, Moore K, Naden P, Nic Aongusa C, Noges T, Weyhermeyer G, 2010. Climate impacts on the flux of dissolved organic carbon from catchments. p. 199-220. In G. Glen, (ed.) *The Impact of Climate Change on European Lakes*. Dordrecht, Heidelberg, London, New York: Springer.
- Jennings E, Jones S, Arvola L, Staehr PA, Gaiser E, Jones ID, Weathers KC, Weyhenmeyer GA, Chiu C, de Eyto E, 2012. Effects of weather-related episodic events in lakes: an analysis based on high-frequency data. *Freshwater Biol.* 57: 589-601.
- Jones HLJ, 1997. A classification of mixotrophic species based on their behaviour. *Freshwater Biol.* 37:35-43.
- Jones RI, 1992. The influence of humic substances on lacustrine planktonic food chains. *Hydrobiologia.* 229:73-91.
- Jones RI, 1994. Mixotrophy in planktonic protists as a spectrum of nutritional strategies. *Mar. Microb. Food Webs.* 8:87-96.
- Jones RI, 2000. Mixotrophy in planktonic protists: an overview. *Freshwater Biol.* 45:219-226.
- Jones SE, Chiu C, Kratz TK, Wu JT, Shade A, McMahon KD. 2007. Typhoons initiate predictable change in aquatic bacterial communities. *Limnol. Oceanogr.* 53:: 1319-1326.
- Jones CG, Samuelsson P, Kjellström E. 2011. Regional climate modelling at Rossby Centre. *Tellus* 63A, 1–3.
- Kelly PT, Solomon CT, Weidel BC, Jones SE, 2014. Terrestrial carbon is a resource, but not a subsidy, for lake zooplankton. *Ecology* 95:1236-1242. doi:10.1890/13-1586.1.

- Kerfoot WC, Budd, JW, Green SA, Cotner JB, Biddanda BA, Schwab David J, Vanderploeg HA, 2008. Doughnut in the desert: Late-winter production pulse in southern Lake Michigan. *Limnology and Oceanography* 53 (2) 589.
- Kritzberg ES, Cole JJ, Pace ML, Granéli W, Bade DL, 2004. Autochthonous versus allochthonous carbon sources to bacteria: Results from whole-lake <sup>13</sup>C addition experiments. *Limnology and Oceanography*, 49(2), 588-596.
- Korolef F, 1983. Simultaneous oxidation of nitrogen and phosphorus compounds by persulphate. In: E. Grasshoff, Ehrhardt, M., Kremling, K. (ed.) *Methods of Seawater Analysis* 2nd Ed. Verlag Chemie, Weinheim, 164-169.
- Kortelainen P, 1999. Occurrence of humic waters. Temporal and spatial variability. p. 46-55. In: J. Keskitalo and P. Eloranta (eds.) *Limnology of humic waters*. Leiden, Backhuys Publishers.
- Kostrzevska-Szlakowska I, Jasser I, 2011. Black box: what do we know about humic lakes? *Pol. J. Ecol.* 59:647-664.
- Karlsson, Jan, Mats Jansson, and Anders Jonsson. "Similar relationships between pelagic primary and bacterial production in clearwater and humic lakes." *Ecology* 83.10 (2002): 2902-2910.
- Laybourn-Parry J, Walton M, Young J, Jones RI, and Shine A, 1994. Protozooplankton and bacterioplankton in a large oligotrophic lake -Loch Ness, Scotland. *J. Plankton Res.*, 16:1655-1670.
- Lund JWG, Kipling C, Le Cren ED, 1958. The inverted microscope method for estimating algal numbers and the statistical basis of estimations by counting. *Hydrobiologia*. 11:143-170.
- Maclsaac EA, Stockner JG, 1993. Enumeration of Phototrophic Picoplankton by Autofluorescence Microscopy. p. 187-197. In P.E. Kemp, B.E. Sherr, E.B. Sherr and J.J. Cole (ed). *Handbook of methods in Aquatic Microbial Ecology*. Lewis Publiser.

- Meili M, 1992. Sources, concentrations and characteristics of organic matter in softwater lakes and streams of the Swedish forest region. *Hydrobiologia*. 229:23-41.
- Mihaljević M, Stević F, Horvatić, J Hackenberger Kutuzović B, 2009. Dual impact of the flood pulses on the phytoplankton assemblages in a Danubian floodplain lake (Kopački Rit Nature Park, Croatia). *Hydrobiologia* 618, no. 1: 77-88.
- Miller MP, McKnight DM, 2010. Comparison of seasonal changes in fluorescent dissolved organic matter among aquatic lake and stream sites in the Green Lakes Valley. *Journal of Geophysical Research: Biogeosciences* 115 (1).
- Montanarella L, Jones RJA, Hiederer R, 2006. The distribution of peat soil in Europe. *Mire and Peat*. 1:2-10.
- Morgan KC, Kalff J, 1979. Effect of light and temperature interactions on growth of *Cryptomonas erosa* (Cryptophyceae). *J. Phycol.* 15:127-134.
- Naden PS, Allott N, Arvola L, Järvinen M, Jennings E, Moore K, Schneiderman E, 2010. Modelling the impacts of climate change on dissolved organic carbon. In *The Impact of Climate Change on European Lakes* (pp. 221-252). Springer Netherlands.
- Nürnberg GK, Shaw M, 1999. Productivity of clear and humic lakes: nutrients, phytoplankton, bacteria *Hydrobiologia*. 38:97-112.
- Obrador, Biel, Peter A. Staehr, and Jesper Philip Aagaard Christensen. "Vertical patterns of metabolism in three contrasting stratified lakes." *Limnology and Oceanography* 59, no. 4 (2014): 1228-1240.
- Ojala A, López Bellido J, Tulonen T, Kankaala P, Huotari J, 2011. Carbon gas fluxes from a brown-water and a clearwater lake in the boreal zone during a summer with extreme rain events. *Limnol. Oceanogr.* 56:61-76.
- OECD (Organisation for Economic Cooperation and Development) 1982. *Eutrophication of Waters, Monitoring, Assessment and Control*. Paris, OECD.

- Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Henry M, Stevens H, Wagner H, 2013. *Vegan: Community Ecology Package* R package version 2.0-10. <http://CRAN.R-project.org/package=vegan>
- Paidere J, Gruberts D, Škute A, Druvietis I, (2007) Impact of two different flood pulses on planktonic communities of the largest floodplain lakes of the Daugava River (Latvia). *Hydrobiologia* 592(1): 303-314.
- Perdue EM, 1998. Chemical composition, structure, and metal binding properties, p. 41-46. In D.O. Hessen and L.J. Tranvik (eds.), *Aquatic humic substances*, vol. 133. Springer, Leiden.
- Poikane S, 2009. Water Framework Directive intercalibration technical report. Part 2: Lakes. IRC Scientific and Technical Reports. EUR 23838 EN/2. Ispra: 178 pp.
- Porcal P, Koprivnjak JF, Molot LA, Dillon PJ, 2009. Humic substances: the biogeochemistry of dissolved organic carbon and its interactions with climate change. *Environ. Sci. Pollut. R.* 16:714-726.
- R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>
- Read JS, DP Hamilton, ID Jones, K Muraoka, LA Winslow, R Kroiss, CH Wu, E Gaiser. 2011. Derivation of lake mixing and stratification indices from high-resolution lake buoy data. *Environmental Modelling and Software.* 26: 1325-1336.
- Riemann B, Havskum H, Thingstad F, Bernard C, 1995. The role of mixotrophy in pelagic environments, p. 87–114. In I. Joint, (ed.), *Molecular Ecology of Aquatic Microbes*. Springer-Verlag, Berlin.
- Rubbo M, Cole JJ, Kiesecker JM, 2006. Terrestrial subsidies of organic carbon support net ecosystem production in temporary forest ponds: evidence from an ecosystem experiment. *Ecosystems.* 9:1170-1176.

- Shaw PJ, Jones, R.I., DeHaan, H.E, 2000. The influence of humic substances on the molecular weight distributions of phosphate and iron in epilimnetic lake waters. *Freshwater Biol.* 45:383–393.
- Sherr EB, Caron DA, Sherr BF. 1993. Staining of Heterotrophic Protists for Visualization via Epifluorescence Microscopy, p. 213-227. In: P.F. Kemp, B.F. Sherr, E.B. Sherr, J.J. Cole (eds.). *Handbook of Methods in Aquatic Microbial Ecology*. Lewis Publ., Boca Raton, FL.
- Smith VH, 1982. The nitrogen and phosphorus dependence of algal biomass in lakes: An empirical and theoretical analysis. *Limnol. Oceanogr.* 27:1101-1112.
- Standing Committee of Analysts, 1983. The determination of chlorophyll-a in aquatic environments 1980, Her Majesty's Stationery Office, London, UK. 26 pp.
- Solomon CT, Bresewith DA, Richardson DC, Rose KC, Van de Bogert MC, Hanson PC, Kratz TK, Larget B, Adrian R, Leroux BB, Chiu CC, Hamilton DP, Gaiser EE, Hendricks S, Istvánovics V, Laas A, O'Donnell DM, Pace ML, Ryder E, Staehr PA, Torgensen T, Vanni MJ, Weathers KC, Zhu G., 2013. Ecosystem respiration: Drivers of daily variability and background respiration in lakes around the globe. *Limnol. Oceanogr.* 58: 849-866.
- Sucker C, Krause K, 2010. Increasing dissolved organic carbon concentrations in freshwater: what is the actual driver? *Biogeosciences and Forestry.* 3:103-108.
- Sun J, Liu D, 2003. Geometric models for calculating cell biovolume and surface area for phytoplankton. *J. Plankton Res.* 25:1331-1346.
- Tarnocai C, 2006. The effect of climate change on carbon in Canadian peatlands. *Global and Planetary Change* 53(4): 222-232.
- Tipping E, 1981. The absorption of aquatic humic substances by iron oxides. *Geochim. Cosmochim. Ac.* 45:191-199.

- Tranvik LJ, 1988. Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of differing humic content. *Microbial Ecol.* 16:311-322.
- Tranvik LJ, 1989. Bacterioplankton growth, grazing mortality and quantitative relationship to primary production in a humic and a clearwater lake. *J. Plankton Res.* 11:985-1000.
- Tranvik LJ, Downing JA, Cotner JB, Loiselle SA, Striegl RG, Ballatore TJ, Dillon P, Finlay K, Fortino K, Knoll LB, Kortelainen PL, Kutser T, Larsen S, Laurion I, Leech DM, McCallister SL, McKnight DM, Melack JM, Overholt E, Porter JA, Prairie YT, Renwick WH, Roland F, Sherman BS, Schindler DW, Sobek S, Tremblay A, Vanni MJ, Verschoor AM, von Wachenfeldt E, Weyhenmeyer GA, 2009. Lakes and reservoirs as regulators of carbon cycling and climate. *Limnol. Oceanogr.* 54:2298-2314.
- Twiss MR, McKay RML, Bourbonniere RA, Bullerjahn GS, Carrick HJ, Smith REH, Winter JG, 2012. Diatoms abound in ice-covered Lake Erie: An investigation of offshore winter limnology in Lake Erie over the period 2007 to 2010. *Journal of Great Lakes Research* 38 (1): 18-30.
- Utermöhl H, 1958. Zur Vervollkommung der quantitative Phytoplankton Methodik. *Verh. Internat. Verein Limnol.* 9:1-38.
- Vadrucci MR, Cabrini M, Basset A, 2007. Biovolume determination of phytoplankton guilds in transitional water ecosystems of Mediterranean Ecoregion. *Transitional Waters Bulletin* 2:83-102.
- Walsh S, 2010 Report on rainfall of November 2009. *Met Eireann Climatological Note* 12. [http://www.met.ie/climate-ireland/weather-events/Nov2009\\_rain.pdf](http://www.met.ie/climate-ireland/weather-events/Nov2009_rain.pdf)
- Weyhenmeyer GA, Willén E, Sonesten L, 2004. Effects of an extreme precipitation event on water chemistry and phytoplankton in the Swedish Lake Mälaren. *Boreal Environ. Res.* 9:409-420.

Worrall F, Burt TP, 2007. Trends in DOC concentration in Great Britain. *Journal of Hydrology*, 346, 81-92.



Table 1 – Physical and chemical characteristics of the two study sites, Lough Feeagh and Lough Guitane. Summary water chemistry characteristics are presented for monthly samples collected between May 2009 and April 2010.

	Feeagh			Guitane		
	Mean	Min.	Max.	Mean	Min.	Max.
Catchment area (km <sup>2</sup> )	89.5			12.04		
Surface area (km <sup>2</sup> )	3.95			2.46		
Max. (average) depth (m)	46 (14.5)			56 (18.7)		
Retention time (days)	164			167		
	Mean	Min.	Max.	Mean	Min.	Max.
pH	6.9	6.7	7.1	6.9	6.7	7.1
Alkalinity (mg L <sup>-1</sup> CaCO <sub>3</sub> )	5.5	4.0	6.0	5.0	4.0	7.0
Conductivity (µS cm <sup>-1</sup> )	82	76	92	49	46	53
Colour (mg L <sup>-1</sup> PtCo)	84	64	110	21	16	26
DOC (mg L <sup>-1</sup> )	7.7	6.2	11.4	3.3	1.5	6.4
TP (µg L <sup>-1</sup> )	8.1	5.0	12	5.3	2.0	15
DMRP (µg L <sup>-1</sup> )	1.6	0.9	3.0	1.1	0.9	3.0
TN (µg L <sup>-1</sup> )	462	200	870	340	210	530
NO <sub>3</sub> -N (µg L <sup>-1</sup> )	70	38	83	113	75	180
Chl- <i>a</i> (µg L <sup>-1</sup> )	1.1	0.2	3.0	2.2	0.8	4.3

## Figure Legends

Figure 1. Location of the two study sites, Loughs Feeagh and Guitane and their catchment boundaries.

Figure 2. Water column temperatures ( $^{\circ}\text{C}$ ) between May 2009 and April 2010 in Feeagh (left) and Guitane (right) at subsurface (2-2.5m – open grey circles), mid water (20m – black filled circles) and at the lake bottom (40-42m (open black triangles).

Figure 3. Seasonal variation in colour, DOC, secchi depth, chlorophyll  $\alpha$ , total phosphorus and total nitrogen in Loughs Feeagh (grey open circles) and Guitane (black filled circles) between May 2009 and April 2010. Arrows indicate the timing of heavy rainfall events in the Feeagh (grey) and Guitane (black) catchments.

Figure 4. Daily rainfall and average daily turbidity measurements from Lough Feeagh between May 2009 and April 2010. Turbidity was measured using an in-situ nephelometer.

Figure 5. Biomasses ( $\text{mm}^3 \text{m}^{-3}$ ) of total openwater plankton, autotrophic phytoplankton, bacteria, potential mixotrophic phytoplankton, ciliates and the phytoplankton:bacteria ratio in Feeagh (grey open circles) and Guitane (black filled circles) between May 2009 and April 2010. Arrows indicate the timing of heavy rainfall events in the Feeagh (grey) and Guitane (black) catchments.

Figure 6. Level of separation in biotic groups sampled from the pelagic zones of Lough Feeagh (F) and Lough Guitane (G). Each black circle represents a different month from May

2009 to April 2010. Dissimilarities were calculated using the Bray-Curtis measure. Arrows indicate vectors of environmental variables significantly correlated with the ordination of biotic variables ( $p < 0.001$ ). The weighted average scores for each biotic group are indicated by the group label (grey). Stress=0.18. Autotrophic and mixotrophic components of groups are denoted with \_a and \_m where appropriate.

Figure 7. Ethanol extracted chlorophyll a ( $\mu\text{g L}^{-1}$ ) measured in openwater samples in Lough Feeagh (top) and Lough Guitane (bottom). The grey shaded areas indicated samples taken between May 2009 and April 2010. The black lines represent long term monthly averages during 2007-2014 for Feeagh and 1999-2008 for Guitane, excluding the period of interest 2009-2010. Error bars indicated the standard deviation in long term averages. Arrows indicate the timing of heavy rainfall events.















