Title:

Assessing the status of shallow lakes using an additive model of biomass size spectra

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ABSTRACT

1. Planktonic biomass size spectra were used to summarise the ecological quality of six shallow lakes sampled in spring, early summer and late summer.

2. A simple additive model fitted to the data was used to assess the applicability of the size spectrum theory to shallow lake ecosystems.

3. The additive model replicated the hierarchical pattern of biomass predicted by the predator-prey theory of aquatic production, and was a more appropriate model for predicting biomass size spectra than the frequently used linear regression.

4. Lakes with varying ecological quality were a significant source of variation in the additive model, and further research into using size spectra to monitor ecological quality in shallow lakes is warranted. Specifically, the production of size spectra from a wider range of sites is needed to provide greater statistical validation.

5. The use of size spectra can provide an attractive and cost-effective way for classifying lake ecosystems because it circumvents the need for difficult taxonomic description.

INTRODUCTION

The usefulness of biomass size spectra in condensing and analysing features of aquatic

ecosystems has been gaining acceptance in recent years, particularly with the realisation

that indices of ecosystem function may be useful in determining and monitoring ecological

quality in aquatic ecosystems. The search for simple and effective descriptors of biological

ecosystem components is a major challenge of monitoring aquatic ecosystem health (Basset

et al., 2004), and is receiving much attention in Europe, in preparation for the

implementation of the Water Framework Directive (WFD – Directive 2000/60/EC of the

European Parliament and of The Council of 23 October 2000 establishing a framework for

Community action in the field of water policy). The WFD places high emphasis on the use

of taxonomy in assessing ecological quality. In the explanation of how biological quality

elements should be used to monitor ecological quality (WFD, Annex 5, section 1.2),

taxonomic composition is mentioned in all cases. Lack of comparability of taxonomic

groups across ecoregions, as well as a lack of expertise and the time consuming nature of

taxonomic work are issues that are likely to be problematic for implementing the Directive.

Article 20 of the WFD provides for the development of alternatives to taxonomic

measurements. The aim of this paper is to assess the use of size spectrum analysis as one

such alternative for monitoring ecological quality in shallow lake ecosystems.

Interest in the use of ecosystem function indices, such as biomass size spectra, has

increased in recent years, both in freshwater and marine systems. Biomass size spectrum

analysis describes the distribution of biomass in progressively increasing size classes. A

biomass size spectrum is constructed by measuring and counting individuals in the relevant

sample(s), converting the measurement to some form of body mass unit (e.g. equivalent spherical diameter, or grams of carbon) and graphing this (x-axis) against some form of abundance unit (y-axis). The first size spectra for oceanic open water plankton (Sheldon *et al.,* 1972) found that total standing biomass in increasing logarithmic size classes was approximately constant. Subsequent studies in a wide variety of open water marine and freshwater communities have shown a generality of a constant biomass distribution for a remarkable range of waterbodies (Gaedke, 1992; García *et al.*, 1995; Witek and Krajewska-Soltys, 1989). This constant nature is indicated by the slope of the normalised biomass size spectrum fitted to a straight line, with a value of -1. The normalised biomass is simply the biomass of organisms within a size range (i.e. the abundance estimate on the y-axis), divided by the width of the body mass interval. Size spectrum theory has undergone much development since the 1970s, and has considerable potential for increasing understanding of aquatic ecosystem trophic structure, as it is the underlying ecological energetics, particularly related to predator-prey interactions, which control the patterns in size spectra (Kerr and Dickie, 2001).

The main advantage of the size spectrum approach is that it condenses a large amount of ecosystem information into essentially one column of numbers, and succinctly summarises the ecosystem of the study site. In addition, it is an ataxonomic approach (Echevarría *et al.*, 1990), in that the data can be collected without having a high level of taxonomic expertise in each of the biological components. Cattaneo *et al.* (1993) have shown that attributes with intermediate levels of detail, such as growth form or size spectrum, may be most efficient for biomonitoring because they explain similar amounts of variance as fine-level taxonomy, but they require less time and taxonomic expertise. Size

spectrum analysis also provides a way of generally comparing ecosystems, which may have quite different species compositions owing to geography. Size spectrum analysis is particularly suited to examining the effects of perturbation on the whole food web of an ecosystem (Cottingham, 1999), and as a result is an increasingly widespread approach in both marine and freshwater science, particularly to monitor exploitation of commercial fish stocks (Graham *et al.,* 2005; Shin *et al.,* 2005).

While models of size spectra have been shown to fit data from large lakes and oceans, they remain relatively untested for shallow lakes, and also ranges of biomass that include microbial elements. It is thought that planktonic biomass size spectra may be more spatially and temporally variable in lakes than in oceans, owing to substantial inputs from surrounding land into a relatively small volume, and to the influence of benthic organisms in the biomass size spectra of lakes. The benthic influence may be particularly important in shallow lakes, where benthic and planktonic food webs are closely linked (Cyr and Peters, 1996). It is also likely that observed switches between alternative steady states in shallow lakes (Scheffer, 1998) may affect the stability of the biomass size spectra.

Obviously, size spectra will only be useful as ecosystem function indicators if they are sensitive to variation among and within lakes. The slope of the normalised size spectrum has been shown to become less negative with increasing lake productivity (Ahrens and Peters, 1991; Sprules and Munawar, 1986) and others factors suggested to affect the shape and slope of the normalised size spectrum include latitude, water depth, and lake size (Gaedke, 1992). These studies indicate that factors affecting the ecological quality of lakes such as nutrient enrichment (the main pressure in many European lakes), acidification and catchment degradation as a result of land use changes are likely to be

reflected in the size spectra. It seems likely, therefore, that graphing and analysing the size spectra of the openwater communities may be a viable alternative to taxonomic monitoring of ecological quality.

In Irish lakes (and hence our study lakes), the primary factor affecting lake ecological quality is nutrient enrichment (Toner *et al.*, 2005). In order for size spectrum analysis to adequately capture changes in trophic status, it must therefore be sensitive to changes in the openwater planktonic food web that come about as a result of increased nutrient loading. Some of these food web changes include a general increase in biomass with increasing lake productivity, reflected in a higher intercept in the normalised size spectrum (Boudreau and Dickie, 1992). Results from previous studies on the response of the phytoplankton assemblage to increased productivity indicate that the proportion of large taxa in the phytoplankton usually increases as lakes become more eutrophic (Cottingham, 1999; Kalff and Knoechel, 1978; Watson and Kalff, 1981), and this should be obvious in the normalised size spectrum as a relatively higher biomass of phytoplankton in the larger size classes. Changes in the zooplankton and fish assemblages with productivity are inextricably linked and are likely to lead to a disruption in the trophic cascade. For example, the loss of planktiviorous fish (e.g. as a result of summer fish kills owing to anoxia) would lead to an increase in the size of cladocera in accord with the size efficiency hypothesise (Brooks and Dodson, 1965). This, in turn, is likely to have implications for both the phytoplankton size distribution, as there is a strong correlation between zooplankton size and phytoplankton mean volume, and also possibly for the microbial part of the food web (Jeppensen *et al.*, 1998). All these changes should be apparent in the size spectrum as shifting domes of biomass.

The first step in assessing whether size spectrum analysis can be used to assess and monitor ecological quality in shallow lakes is to determine whether size spectra vary across a range of lakes of varying ecological quality. This study aims to address this question by producing size spectra for plankton in 6 Irish lakes of varying quality (Moss *et al.,* 2003). As seasonality may play a large role in structuring the slope and shape of the size spectrum (Gaedke, 1992; Tittel *et al.*, 1998), the lakes were sampled in spring, early summer and late summer. Normalised size spectra of the open water plankton, ranging from bacteria to predatory cladocera, were constructed for each lake for late spring (April), early summer (June) and late summer (August). We wanted to ascertain whether there was commonality in size spectra across a range of shallow lakes, and determine whether size spectrum models are sensitive to trophic, physicochemical or seasonal variation.

Analysis of size spectra of freshwater ecosystems has largely been based on fitting the data to a straight line (e.g. (Gaedke, 1992; García *et al.*, 1995; Sprules and Munawar, 1986; Tittel *et al.*, 1998). Several other models have, however, been developed for describing size spectra. These include the predator-prey size spectrum model summarised in Kerr and Dickie (2001), models based on pareto distributions (Brucet *et al.,* 2005; Quintana *et al.,* 2002), and models based on the precept that the trophic structure of aquatic communities is closely related to organism size (Borgmann, 1982; Borgmann, 1987). Rather than confine our analysis to previously published models, which may or may not be appropriate to shallow lake ecosystems, we used additive modelling to find the best fit for the data.

METHODS

Study sites and field sampling - The six lakes chosen for this study (Carra, Gara, Gur, Maumwee, Mullagh, Ramor) cover a range of the physicochemical and ecological conditions found in Ireland (Table 1). In addition, Loughs Carra and Maumwee are both situated in designated SACs (Special Areas of Conservation). Lough Carra is part of the Lough Carra/Mask Complex SAC, with the lake itself designated as the Habitats Directive (92/43/EEC) habitat 'Hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp' (HD code 3140). Lough Maumwee is in a large SAC complex (Maumturk Mountains SAC) and the lake is designated as 'Oligotrophic waters containing very few minerals of sandy plains (*Littorelletalia uniflorae*)' (HD code 3110). Both of these lakes may require additional monitoring as protected areas under the WFD.

The lakes were sampled in April, June and August 2000. Composite water samples were collected on each sampling occasion from the deepest point of each lake using a 3 metre plastic tube. In lakes with a maximum depth of less than 3 metres, the water samples were taken at 30-50 cm depth, from the boat. Subsamples of these water samples were preserved with Lugol's iodine for phytoplankton and ciliate counts, and with Formalin (final concentration 1.5%) for counts of bacteria, heterotrophic nanoflagellates (HNF) and autotrophic picoplankton (APP). The rest of the water was used for chemical analysis. Zooplankton were sampled by vertical hauls of the water column using a conical zooplankton net (53 µm mesh, with a flow meter for correcting filtering efficiency). Where the lake was too shallow for effective use of the net, 10 litres of water was collected using a

perspex tube (diameter 5.5 cm, volume 2276 cm^2). Samples were preserved in excess 70% ethanol.

Chemical analysis and biomass determination - Total phosphorus (TP) was determined using potassium persulphate digestion, followed by the spectrophotometric molybdenum-blue method of Eisenreich *et al.* (1975). Chlorophyll *a* was determined using ethanol extraction according to ISO 10260 (1992). Total N (TN) was determined in duplicate by alkaline persulfate digestion followed by flow-injection analysis (Tecator Kjeltec Analyzer System), which involved Cd reduction followed by azo dye colorimetry. Alkalinity was determined by titration with H_2SO_4. Colour was measured at an absorbance of 400nm. Conductivity, turbidity and pH were determined using electronic meters. Ecological quality was assigned using the ECOFRAME methodology developed in Moss *et al*. (2003), which was based on a combination of 28 variables comprising biological, physical and chemical data.

To count and measure bacteria, HNF and APP, aliquots were filtered onto black 0.22µm Isopore filters, stained with DAPI (4'-6-Diamidino-2-phenylindole) and counted using epiflouresence, following the methodologies of Porter and Feig (1980) and Kemp *et al.* (1993). Digital photographs of the fields of view at x1000 magnification were taken and individuals were counted and measured using the computer programme Scion Image, version 4.0.2. Phytoplankton, ciliates and rotifers were counted and measured using an inverted microscope (x400 magnification) and an eyepiece graticule. Zooplankton samples were subsampled using a 5 ml wide bore pipette, and counted and measured using a dissecting microscope (x30). Individual measurements were converted into biomass (pg

Carbon) using the conversion factors in Gaedke (1992). The body size of bacteria, APP,

HNF, phytoplankton and ciliates were calculated from standard geometric formulas.

Colonial phytoplankton were treated as individuals only in cases where it was too difficult

to differentiate between cells as they shared a common surface area. In general, therefore,

the individual cells of the colony were counted and measured. Measurements of rotifers

were converted to dry weight using Latja and Salonen (1978) and Telesh *et al.* (1998).

Measurements of the larger crustaceans were converted to dry weights using length-weight

regressions calculated for various species from each lake during the course of the study (de

Eyto and Irvine, 2005).

Statistical analysis – Initially, size spectra were constructed for each lake and each

sampling period, using $\log_2$ (pg C) size classes. Normalised size spectra were produced for

the whole range of sizes from bacteria to predatory cladocerans. To produce the normalised

size spectra, the biomass in a size class was divided by the width in picograms of carbon of

that size class. Normalised size spectra were analysed using additive modelling in the

computer package Brodgar v 2.4.3 (www.brodgar.com). The response variable (normalised

biomass) was modelled using size class, month and lake as explanatory variables. Size class

was included as the smoothing term, while month (1 – April; 2 –June; 3 -August) and lake

(1- Carra; 2 – Gara; 3 – Gur;, 4 – Maumwee; 5 – Mullagh;, 6 – Ramor) were included as

nominal variables. The optimal degrees of freedom of the smoothing term was found using

cross-validation (Wood, 2000; Wood, 2004). An additive model was used rather than a

simple linear model as the residuals from a linear model showed distinct patterns or clumps

of both negative and positive residuals (Fig. 1). Including interaction terms in the linear

model did not reduce the patterns in the residuals, indicating the relationship between size class and normalised biomass was non-linear. The spread of the residuals of the additive model with a Gaussian distribution was homogenous, indicating that there was no requirement to use a generalised additive model with a Poisson distribution (Montgomery *et al.*, 2001; Zuur *et al.*, 2006 (In Press)). An interaction term between month and lake was included in the model. F-tests were used to test the significance of the interaction and smoothing terms.

RESULTS

The ECOFRAME scheme for assessing ecological status (Moss *et al.,* 2003), classified Loughs Gara, Gur and Mullagh as having moderate ecological status, Carra and Maumwee as high, and Ramor as poor. This was based on 80% compliance for all variables in the scheme. The size spectra of the plankton communities of the six lakes extended over 34 $\log_2$ body size classes, with individual biomasses ranging from 0.0078 pg C (bacteria and autotrophic picoplankton) to 6710864 pg C (predatory cladocera). Total openwater planktonic biomass ranged from 0.08µg C per ml in Lough Gara in April to 1.47µg C per ml in Lough Mullagh in August. Generally, Lough Ramor and Lough Mullagh had the highest biomass, Lough Gara and Lough Maumwee the lowest, with Lough Carra and Lough Gur intermediate between the two. A full description of the plankton communities, and the relative contribution of each functional group to the total biomass can be found in de Eyto and Irvine (2005).

The size spectra of the openwater plankton showed a decreasing pattern with several apparent domes of biomass (Fig. 2). The optimum additive model confirmed the presence

of these domes, with the smoothing term having 8.31 estimated degrees of freedom (Fig. 3) with an approximate significance of $p<0.001$. In the additive model, lake was a significant source of variation for the normalised biomass ($p<0.001$), as was the interaction term *month x lake* ($p<0.001$). The normalised biomass did not vary significantly among months ($p=0.08$). A summary of the optimum additive model is given in Table 2.

Examination of the parametric coefficients for each lake and month indicated how the size spectrum model changed according to lake and *month x lake* (Table 3). The intercept of the model was significantly lower (i.e. the whole curve shifted down) for Lough Gara ($p=0.008$) than for Lough Carra, while those of Lough Mullagh and Ramor were significantly higher (i.e. the whole curve shifted up) ($p<0.001$ in both cases). This is apparent in Fig. 4, as there are many points representing Lough Gara below the fitted curve towards the right side of the graph, while several of the points (in the range of larger phytoplankton taxa) representing Loughs Mullagh and Ramor are above the curve in the middle of the graph. The intercept was generally related to the total biomass of the openwater plankton for each group, with more productive lakes having higher intercepts. Given the small dataset, it would misleading to investigate significant correlations between the model and physicochemical variables. However, there is a general trend of increasing intercept with increasing productivity, as indicated by total phosphorus, chlorophyll *a* and turbidity, and decreasing intercept with increasing water hardness (as indicated by conductivity, alkalinity and pH) (Fig. 5).

The interaction between month and lake was also a significant source of variation, particularly for Lough Gara in August, which had a significantly higher intercept

($p=0.008$), than in other months, while Lough Ramor in June had a significantly lower intercept ($p=0.015$) than in other months.

DISCUSSION

The lakes that the additive model highlighted as having different size spectra from Lough Carra (high ecological quality) were Loughs Mullagh and Ramor (classified under ECOFRAME as moderate and poor quality, respectively) and Lough Gara (moderate). The other lake of moderate quality (Lough Gur) did not have a significantly different size spectrum than Lough Carra, although the p value was quite small ($p=0.10$). The two lakes with the most similar size spectra were Loughs Carra and Maumwee ($p=0.54$), which are both classified as high quality according to the ECOFRAME scheme. These two lakes have distinctly different alkalinity and, therefore, would be placed into different water body types under WFD. As a first step, this indicates that size spectrum analysis may be appropriate for monitoring ecological quality. The ECOFRAME method of assessing ecological quality is, for the most part, independent of the data that was used to construct the size spectra, yet the size spectra results mirrored the ECOFRAME classifications reasonably well. The biological variables in the ECOFRAME scheme included the number of phytoplankton species, the proportion of large cladoceran species in the zooplankton and the ratio of zooplankton to phytoplankton, all of which would contribute to the biomass size spectra. However, the ECOFRAME scheme comprised 25 other variables unrelated to the biomass size spectra.

The time of year (month) was not a significant source of variation for the additive

model, which indicates that the size spectra are relatively stable within the year. There were

two exceptions: the size spectra of Lough Gara in August, and Lough Ramor in June, were

different from other months.   In Lough Gara in August, there was a general increase in

biomass of both bacteria and zooplankton compared with other months, causing the whole

curve to shift slightly upwards. In Lough Ramor, the different size spectra in June reflected

a much lower zooplankton dome than in the other two months. Total zooplankton biomass

in Lough Ramor in June was only 0.014 µg C ml-1, in comparison with 0.35 and 0.12 µg C

ml-1 in April and August respectively (de Eyto and Irvine, 2005). This may be owing to the

appearance of *Leptodora kindtii*, which was not present in the other two months. The

presence of this large predatory cladoceran caused the zooplankton dome to move slightly

to the right of the graph (bigger size class), and plays a crucial role in the structure of the

size spectrum; causing the intercept of the additive model to decrease significantly by –

1.93.

Trophic state of the lakes was reflected in size spectrum analysis. Firstly, more

productive lakes had higher intercepts, indicative of a general increase in biomass, in

accord with the predictions of Boudreau and Dickie (1992). Secondly, the two most

productive lakes (Mullagh and Ramor) had a higher proportion of large taxa in the

phytoplankton (Fig. 4). As we did not include fish in this analysis, it is difficult to interpret

differences in the zooplankton assemblage as a result of nutrient enrichment, as the two are

so closely linked through top down control. In addition, the fish assemblages of the six

lakes vary considerably, and hence will have different impacts on the zooplankton

assemblage. For example, Lough Carra is a brown trout (*Salmon trutta*) fishery (King and

Champ, 2000), with extensive *Chara* beds that provide excellent refuge for large cladocerans. In contrast, the fish assemblage of Lough Gur is dominated by rudd (*Scardinius erythrophthalmus* L.) and also has some pike (*Esox lucius* L.) and eel (*Anguilla anguilla* L.) (King and O'Grady, 1994). Nevertheless, the two brown trout lakes (Carra and Maumwee) both had similar size spectra, despite their chemical differences, and that these differed significantly from Loughs Mullagh, Ramor and Gara, which are predominantly coarse fisheries. This observation underpins the link between top down control by fish, and the general ecological quality of a lake, and highlights the fact that, in Ireland at least, there is a strong correlation between nutrient enrichment and lakes switching from salmonid to coarse fisheries.

Size spectrum models based on mathematical formulations describing energy transfer, production and body size ratios between predators and prey predict a hierarchical structure in the size spectra (Thiebaux and Dickie, 1993) as predator–prey relationships lead to powerful size-based trophic structuring (Shin *et al.,* 2005). This predator-prey model predicts that over a wide range of body sizes, a smooth parabola with a very low negative curvature may be the best fit for biomass size spectra. In addition, it predicts that there is some degree of secondary scaling, with groups of predators and their prey appearing as periodic oscillations or domes of biomass. Thiebaux and Dickie (1992; 1993) predicted that if these domes are fitted to parabolas, and if a fixed R (predator – prey size ratio) is presumed, then the smooth quadratic integral spectrum can be overlaid with parabolas representing approximate trophic positions, with each parabola having fixed curvature and uniform vertical and horizontal displacement among functional groups. The best fit of the additive model contained a smoothing curve with four apparent 'domes' of

biomass. This is particularly noticeable in the size classes between –7 and 0, and between 18 and 26. Two other domes occurred between size classes 0 and 8, and 8 and 16, although they are less pronounced (Fig. 3). The first dome was within the size classes of bacteria and the smaller phytoplankton taxa. The second dome comprised HNF, some smaller ciliates and mid-sized phytoplankton. The third dome comprised larger ciliates, rotifers and the larger phytoplankton taxa (as well as colonial phytoplankton). The last dome primarily comprised copepod and cladoceran zooplankton. While we did not attempt to fit these specific parabolic equations used by Kerr and Dickie (2001), our additive model still highlighted these apparent peaks and troughs of biomass, with horizontal shifts between domes of about 8 size classes ($\log_2$ size classes pg C) and vertical shifts between normalised biomass of domes of about 7 ($\log_2$ pg C ml^{-1} / width of size class). The predator-prey theory of biomass size spectra suggests that these are representative of predator-prey couplings, and indicate the trophic position of each group. Our data support the view that there are uniform horizontal shifts among trophic groups if there is a fixed ratio between predators and prey ratio, and that the predator-prey model, as summarised in Kerr and Dickie (2001), is applicable to shallow lake ecosystems. This extends the range of aquatic systems to which this theory may be applied. That the model fitted well to data from small shallow lakes, which are likely subject to high seasonal fluctuation and localised impact from catchment, and have strong linkages with the benthos, strengthens the view that the model is applicable to many aquatic environments.

Our analysis suggests that it is inappropriate to use straight lines to model biomass size spectra, as one of the main assumptions of linear regression is violated, i.e. that

residuals should not show any patterns (linearity). This means that R^2 values and the slopes of the line are unreliable if the model used is a linear regression.

We support the view that biomass size-spectra provides a useful tool for summarising and condensing information about ecosystems into easily analysed models, and our data suggest that the resulting models are sensitive to variation in ecological quality. The use of simple descriptors of biological ecosystems, such as body size distributions, for monitoring purposes is receiving increasingly more interest as anthropogenic influences continue to impact on freshwaters (Basset *et al.,* 2004), and the increasing use and awareness of AM (additive modelling) and GAM (general additive modelling) should make the analysis and use of biomass size spectra more accessible and statistically viable. In addition, the development of automated counting and measuring systems will make it cost-effective to construct biomass size spectra in the future. While we have shown that size spectra vary significantly among lakes of differing ecological quality, we are aware that in this study, ecological quality and the variance in size spectra, is mainly related to eutrophication, and based on rather a small sample size. Further research into how size spectra might vary with other pressures and impacts, including changes in fish assemblages is required. In addition, analysis of size spectra across a wider range of lakes, or within the same lake undergoing ecological change would be useful in developing and quantifying appropriate size spectra metrics for monitoring use.

The use of size-spectra in assessment of ecosystem health may be particularly important for the monitoring of protected areas, including those designated under conservation legislation. These sites merit a greater intensity of monitoring because of their high national or international importance. Size-spectra analysis provides cost-effective

information that can span all, or most, trophic levels; thereby providing a metric encompassing the overall ecosystem. Shifts in size spectra can elicit the need for further investigation. Furthermore, it may be especially useful for a surrogate of the status of fish populations where labour-intensive monitoring may be expensive or unreliable.

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FIGURE LEGENDS

Figure 1. Residual pattern generated when a linear regression is fitted to normalised biomass size spectra 6 Irish shallow lakes. The solid line is a smoothing curve showing the relationship between each x value and the corresponding residual, and the dashed lines represent 95% confidence intervals for the relationship. Size class is measured in units of Log_2 body mass (pg C).

Figure 2. Normalised biomass size spectra for 6 Irish shallow lakes sampled in April, June and August (18 data sets in total). The size ranges of each taxonomic group are shown. Size class is measured in units of Log_2 body mass (pg C).

Figure 3. Additive model smoothing curve fitted to the normalised biomass of openwater zooplankton sampled from 6 shallow Lakes. Dashed lines represent 95% confidence intervals around the main effects. Size class is measured in units of Log_2 body mass (pg C).

Figure 4. Scatterplot with fitted AM (additive model) smoothing curves for the relationship between size class (log_2 pg C) and the normalised biomass of openwater plankton of six shallow lakes. Size class is measured in units of Log_2 body mass (pg C).

Figure 5. Relationship between the intercepts for 6 lakes as predicted by the additive model with TP (left) and Alkalinity (right). Values of TP and alkalinity are averages of three sampling occasions.

Table 1. Characteristics and morphometric data for 6 Irish lakes in this study. Trophic status is classified according to the modified version of the O.E.C.D. classification scheme (1982) used by the Irish EPA (Lucey *et al.*, 1999) according to maximum values of Chlorophyll *a*. Catchment compostion is assigned based on the dominant geology (>50%) in the catchment.

Lake (basin)	Position (Lat Long)	Mean depth (m)	Max. depth (m)	Catchment composition	Annual time (yrs)	Area (Ha)	Trophic status
Carra (north)	53º 42' N 09º 15' W	2	16	Calcareous	0.2	1500	Oligotrophic
Gara (south)	53º 55' N 08º 27' W	1	1.6	Calcareous	0.02	202	Mesotrophic
Gur	52º 31' N 08º 32' W	1.5	3.8	Calcareous	0.21	78	highly Eutrophic
Maumwee	53º 28' N 09º 32' W	2	7.9	Organic	0.1	27	Oligotrophic
Mullagh	53º 49' N 06º 57' W	2.3	8.1	Siliceous	1.34	35	highly Eutrophic
Ramor	53º 49' N 07º 04' W	3	5.5	Siliceous	0.17	741	Hypertrophic

Table 2. Summary of optimum additive model. A normal (gaussian) distribution was assumed for the response variable (normalised biomass). AIC (Akaike Information Criterion) is a measure of fit for a model.

		df	Chi sq	P
Parametric terms	Month	2	5.02	0.0821
	Lake	5	102.93	<0.001
	Month x lake	10	29.53	0.0012
Smoothing terms	s (Size class)	8.84 (est.)	9766.9	<0.001
Model parameters	Deviance explained	95.7%		
	Dispersion	4.47		
	AIC	2446.32		

Table 3. Parametric coefficients calculated for an additive model for normalised biomass of openwater plankton in 6 shallow lakes, sampled three times. The model comprises a smoothing curve (size class) and two nominal variables, lake (n=6) and month (n=3). Lough Carra and April are the baselines for the model. Significantly (95%) different values are in bold.

Variable		Estimate	Standard error	t ratio	*p*
Intercept		1.82	0.41	4.45	**<0.001**
Month	June	0.78	0.57	1.38	0.168
	August	1.29	0.58	2.22	**0.026**
Lake	Garra	-1.52	0.57	-2.65	**0.008**
	Gur	0.94	0.58	1.62	0.105
	Maumwee	0.36	0.59	0.62	0.538
	Mullagh	2.24	0.57	3.95	**<0.001**
	Ramor	3.71	0.57	6.47	**<0.001**
Month x lake	June x Gara	1.39	0.81	1.72	0.087
	August x Gara	2.15	0.81	2.64	**0.008**
	June x Gur	-0.69	0.81	-0.86	0.392
	August x Gur	0.17	0.81	0.21	0.831
	June x Maumwee	-1.00	0.82	-1.22	0.224
	August x Maumwee	-1.13	0.82	-1.38	0.169
	June x Mullagh	-0.19	0.80	-0.24	0.811
	August x Mullagh	0.21	0.80	0.25	0.799
	June x Ramor	-1.93	0.80	-2.42	**0.016**
	August x Ramor	-1.16	0.82	-1.41	0.158