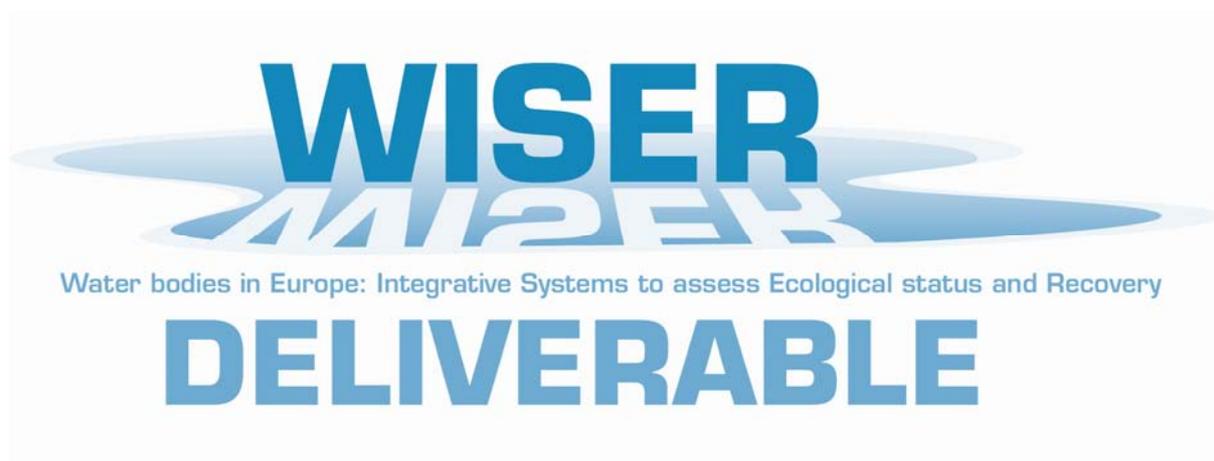


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**Deliverable 3.4-2:** Changes in size structure of fish assemblages in European lakes along eutrophication and hydromorphological pressure gradients.

**Lead contractor:** Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), Berlin

**Contributors:** Matthias Emmrich, Sandra Brucet, Ian J. Winfield, Erik Jeppesen, Torben L. Lauridsen, Pietro Volta, Christine Argillier, Stéphanie Pedron, Kerstin Holmgren, Trygve Hesthagen, David Ritterbusch, Thomas Mehner

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PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

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## **Non-technical summary**

Measurement of ecological integrity using fish fauna is widely applied in the monitoring of freshwater ecosystems, including lakes. According to the European Water Framework Directive fish fauna has to be assessed, via analyses of the composition, abundance and age structure. However, aging of fish is time-consuming and expensive, whereas analyses of the size structure, which can be used as a surrogate for the age structure, is more feasible because size of fish caught by multi-mesh gillnets is in general recorded during field campaigns. Furthermore, analyses of the size structure of lake fish assemblages can be a promising tool to develop metrics that are comparable across large geographical scales, because differences in fish species composition which can be substantial across Europe have not to be taken into account.

In a preliminary analyses on a small geographical scale (78 lakes in northern Germany) we tested the suitability of non-taxonomic size metrics derived from fish catches by multi-mesh gillnets as a tool for elucidating systematic shifts in lake fish assemblages along gradients of environmental factors (lake size and depth, nutrient status) and lake-use intensity (influence of anthropogenic shore structures, boating, bathing). Several size metrics were correlated to gradients of nutrient concentration, lake area and depth as well as variables related to the proportion and size of predatory fishes in the lakes suggesting size metrics as a useful tool to assess the ecological status of lakes at regional scale.

We then selected sensitive size metrics from the first study and applied them to fish catches by benthic multi-mesh gillnets in 728 lakes distributed over eight European countries. Size diversity, geometric mean length and the maximum length of fish caught was analysed in relation to the lake's geographical location, climate, trophic status and intensity of hydrological and shoreline modifications. However, at a large scale, the size structure of lake fish assemblages was primarily influenced by temperature. Warm lakes were dominated by small-sized fish, whereas in cold lakes the relative proportion of large-sized fish increased. Variables indicating anthropogenic pressures such as eutrophication (total phosphorus concentration) or shoreline-bank modifications were not important at a large scale and most likely overridden by temperature effects. We further showed that all size metrics except geometric mean length were sensitive to the sampling effort (number of nets used), suggesting a minimum of at least ten benthic multi-mesh gillnets per lake required for an unbiased comparison of the size structure of fish assemblages.

The size structure of lake fish assemblages did not respond to the level of anthropogenic pressures found in the European lake ecosystems when compared at a large geographical scale. However, at small geographical scale they indicated differences in intensity of eutrophication.

## 1) Local study (northern Germany)

### 1.1 Introduction

Size is a key property of organisms affecting almost all aspects of their life history and ecology, such as respiration, ingestion, reproduction and life span (Peters, 1983; Calder, 1984). Owing to fast growth during ontogeny and substantial size differences between ontogenetic stages, many populations, including fish, are strongly size structured (Werner & Gilliam, 1984; Post *et al.*, 1999; Blanchard *et al.*, 2009). However, the size structure of populations can be further shaped by biotic interactions, such as predation and competition, and by factors including productivity or physical attributes of the habitats (Persson *et al.*, 1991; Wellborn *et al.*, 1996). Accordingly, size-based analyses can be useful in describing communities and can help to identify the complex effects of biotic and abiotic influences (Strayer, 1991; De Leeuw *et al.*, 2003; Brucet *et al.*, 2010).

Pioneered by Sheldon *et al.* (1972), analyses of size distributions have been applied to various fields of aquatic research, primarily targeting plankton (e.g. Sprules & Munawar, 1986; Gamble *et al.*, 2006). Furthermore, impacts of fisheries on marine fish stocks have been documented by size-spectra analyses (e.g. Rochet & Trenkel, 2003; Sweeting *et al.*, 2009). Petchey & Belgrano (2010) suggested that similar systematic changes in size spectra, as observed for exploited marine fish stocks, occur in other systems under different types of environmental pressures. Indeed, although size-based approaches have been less frequently applied to freshwater fish assemblages, the few existing studies suggest that size structures can adequately characterise systematic shifts between lake fish assemblages along the gradients of water quality (Holmgren & Appelberg, 2000; De Leeuw *et al.*, 2003).

Assemblages of lake fish in Europe are characterised by low regional species richness and low species variability between lakes and are in general dominated by only a few generalist species that are widespread along nutrient gradients (Tonn *et al.*, 1990; Tammi *et al.*, 2003). Recent analyses of lakes within the European Central Plain ecoregion have revealed that only a low amount of taxonomic variability (species diversity and relative species abundance) was related to morphometric, human-use intensity and geographical variables (Mehner *et al.*, 2005; 2007). Furthermore, the originally well established conceptual model describing a gradual succession of lake fish assemblages from a numerical dominance by Salmoniformes to a dominance of perch (*Perca fluviatilis* L.) and finally cyprinids along an increasing nutrient gradient in Europe has recently been challenged by contrary evidence from fish assemblages in Germany (Diekmann *et al.*, 2005; Mehner *et al.*, 2005), Sweden (Holmgren & Appelberg, 2000) and Finland (Olin *et al.*, 2002). Accordingly, the taxonomic

composition of fish assemblages has been shown to be insensitive to the ecological status of lakes in Europe. Therefore, we investigated whether non-taxonomic analyses of size structure can detect shifts in the structure of fish assemblages along gradients in German temperate lakes characterised by a depauperate regional fish-species pool.

Several empirical studies have shown substantial differences in the size structure of fish assemblages in lakes, with a shift towards smaller individuals in more eutrophic systems (Jeppesen *et al.*, 2000; Godlewska & Świerzowski, 2003) and with a higher proportion of large fish in large and deep systems (Holmgren & Appelberg, 2000; Beier, 2001; Wilde & Pope, 2004; Arend & Bain, 2008). In addition to morphometric and chemical characteristics of lakes, the taxonomic and functional differences between lake fish assemblages can also modify their size structures. Persson *et al.* (1991) and Mehner (2010) demonstrated that the size of prey available was positively coupled with the proportion and size of predators in assemblages. Additionally, stunted growth, the plastic response of fish to unfavourable environmental conditions and predominantly observed in discrete systems such as lakes (Roff, 1992), is known for many freshwater fish families including species most frequently occurring in European lakes (see Ylikarjula *et al.*, 1999 and references therein). These empirical observations indicate that both abiotic and biotic factors shape the size structure of fish assemblages. However, a comprehensive comparison of size-related variables of fish assemblages in response to both biotic and abiotic gradients is still missing (Jennings *et al.*, 2001; Griffiths, 2006).

The aim of our study was to test the applicability of non-taxonomic, size-related variables as a tool to elucidate systematic shifts in lake fish assemblages along environmental and lake-use intensity gradients by analysing catch data from standardised surveys by multi-mesh gillnets. In particular, we combined several size-related variables, originally derived from plankton and marine fisheries research, to compare (i) their consistency and variability when applied to multi-mesh gillnet catches of fish in lakes, (ii) their intercorrelations and hence inherent redundancy and (iii) their correspondence with descriptors of lake morphometry and productivity, lake-use intensity and taxonomic and functional classifications of fish assemblages.

## **1.2 Methods**

### **1.2.1 Fish sampling**

The data set comprised 78 lakes, including the 65 lakes analysed by Mehner *et al.* (2005). The lakes are located in the north German lowlands, ecoregion 'Central Plains' (Illies, 1978) (Fig. 1). The fish assemblages were sampled according to the European standard for gillnet

surveys EN 14757 (CEN (European Committee for Standardisation), 2005) between 2001 and 2009.

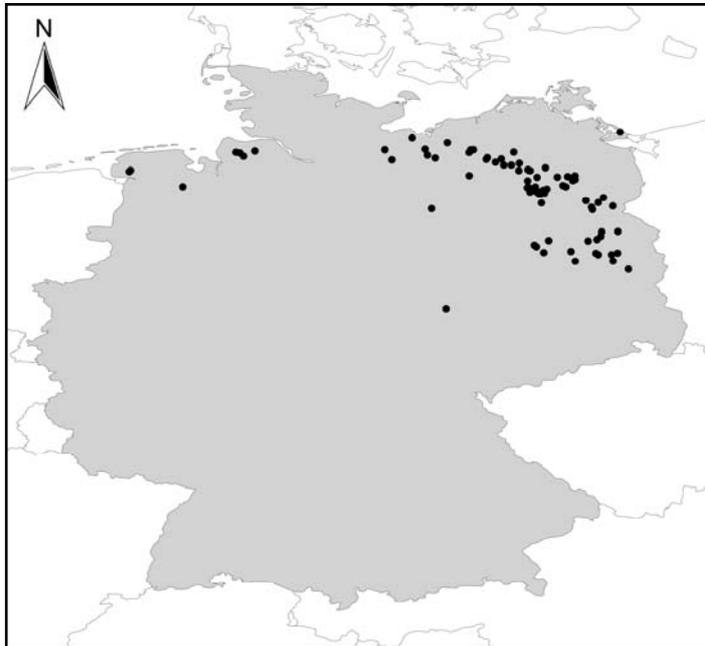


Fig. 1: Geographical location of the 78 natural lowland lakes in Germany (grey coloured), whose fish assemblages were sampled with multimesh gillnets.

The sampling procedure for 55 of the lakes differed slightly from the standard protocol, because for those lakes, the sampling effort was split with the first half of the nets set during late summer or early autumn and the second half set during the subsequent spring (*cf.* Mehner *et al.*, 2005). Each lake was divided into depth strata according the European standard, and each stratum was randomly sampled by a pre-defined number of benthic gillnets (type NORDEN: length 30 m; height 1.5 m; 12 panels of 2.5 m each with mesh sizes (knot to knot) of 5, 6.25, 8, 10, 12.5, 15.5, 19.5, 24, 29, 35, 43 and 55 mm), depending on lake area and maximum depth (Appelberg, 2000; CEN, 2005). Deep lakes (maximum depth > 6 m) were additionally sampled with a row of pelagic nets [similar type as the benthic ones, but of 3 m height and 27.5 m length (5 mm mesh panel missing)] placed over the deepest location in each lake. In the case of complete repeated surveys in the same lake over several years, only data from the most recent sampling campaign were included. Fish were measured (total length, TL) to the nearest cm and weighed (fresh mass, FM) to the nearest gram. Total unweighted catches from both benthic and pelagic habitats were incorporated in the analyses, independently of the relative contribution of benthic and pelagic catches to the total.

Ideally, analyses of catch data should be restricted to size classes for which the catch efficiency of the gear is maximised (Jennings & Dulvy, 2005) and consistent (Rochet & Trenkel, 2003). As young-of-the-year fish are usually underestimated in multi-mesh gillnet catches (Olin & Malinen, 2003; Olin *et al.*, 2009; Prchalová *et al.*, 2009), we excluded all

individuals < 8 cm TL from the analyses. No correction was applied to large fish. Catch efficiency of multi-mesh gillnets for large size classes has not yet been investigated in detail but is assumed to result in only slight overestimates (Prchalová *et al.*, 2009), as catchability does not change strongly with increasing size (Kurkilahti & Rask, 1996). The widely applied standardised norm EN 14757 (CEN, 2005) for sampling lake fish assemblages provides a reliable basis for comparing the size distribution of fish assemblages. Single sample occasions provide an unbiased picture of the size structure of lake fish assemblages (Holmgren, 1999; Holmgren & Appelberg, 2000) and the relative size structure of the gillnet catches did not suffer from the saturation effect, which can underestimate relative fish abundance (Olin *et al.*, 2004; Prchalová *et al.*, 2011).

### 1.2.2 Size-related variables

In total, 18 size-related variables derived from 14 size based approaches (listed below) were calculated from the gillnet catches (Table 1). For the calculation of linearised size spectra, gillnet catches were standardised by calculating the average number of fish per net within each size class (catch per unit effort, CPUE). The pelagic nets (3 m in height and approximately double the area of benthic ones) were considered as two nets for the calculation of CPUE. For all other size-related variables, no catch standardisation was necessary, because the size-related variables and the number of nets or the number of fish caught were only weakly correlated (Spearman's  $r < |0.28|$ , resp.  $r_s < |0.43|$ ).

(1) The geometric mean fish length [ $L_{\text{gmean}}$  (cm)] and (2) variance ( $L_{\text{var}}$ ) (based on length data) were calculated for the entire catch. We further compared (3) the skewness ( $L_{\text{skew}}$ ) and (4) kurtosis ( $L_{\text{kurt}}$ ) of the length–frequency distributions (LFD) (1-cm class intervals). We selected the 99.9<sup>th</sup> percentile (fish length data from all lakes pooled) as the upper length limit (50 cm) to reduce the number of zeros in the LFD. Frequencies were  $\log_{10}(x + 1)$ -transformed. (5) The number of size classes (n SC) with at least one individual (1-cm intervals) was counted, and (6) the interquartile range [IQR (cm)] (the difference between the third and first quartiles), *i.e.* the size range including 50% of all individuals from the catch, was calculated. Then, (7) the maximum length [ $L_{\text{max}}$  (cm)] was selected from the largest individual caught in the gillnets in each lake, and (8) we selected the 95<sup>th</sup> percentile of the LFD [ $L_{95}$  (cm)] as a size variable. Next, (9) the proportion of fish above 'quality length' [ $L_{\text{qual}}$  (%)] included the proportion of fish exceeding the minimum size limit for anglers. We used the median minimum size limit of the five German federal states in which the 78 lakes had been sampled. For species with no minimum size limit, the quality length threshold was set to 30 cm TL. Fulton's condition factor (10) was calculated for each fish separately ( $K_i$ ) by:

$$K_i = \frac{W}{L^3} \cdot 100 .$$

The condition factor of the total community ( $K_c$ ) was calculated as the arithmetic mean of  $K_i$ :

$$K_c = \frac{\sum_{i=1}^n K_i}{n}$$

Furthermore, (11) size diversity (SD;  $\mu$ ) was calculated for each lake following the nonparametric approach of Quintana *et al.* (2008), which is related to the Shannon diversity index but adapted for continuous variables (herein fish total length) corresponding to the integral form:

$$\mu = -\int_0^{+\infty} p_x(x) \log_2 p_x(x) dx$$

where  $p_x(x)$  is the probability density function of the length of each individual fish.

$P_x(x)$  was calculated using nonparametric kernel estimation by:

$$\hat{\mu}_{\text{kerMC}}(X) = \bar{y} - \frac{1}{n} \sum_{k=1}^n \log_2 \left[ \frac{1}{n\sqrt{2\pi}\sigma} \sum_{j=1}^n \exp\left(-\frac{1}{2} \frac{(y_k - y_j)^2}{\sigma^2}\right) \right]$$

Length data were standardised beforehand by division by the sample geometric mean value (Quintana *et al.*, 2008). We also calculated (12) normalised length spectra (NLS), according to Sprules & Munawar (1986) that were modified to fish-length data. We used  $\log_2/2$  size classes to ensure a sufficiently high number of size classes ( $n = 7$  covering a length range of 8–91 cm). All fish > 91 cm TL ( $n = 5$ ) were allocated to the largest class (64–91 cm). The fish in each class were divided by the number of nets and by the width of the size class and were subsequently  $\log_2(x + 1)$ -transformed. The transformed abundances were plotted against the  $\log_2$  midpoints of each size class, and the slope and coefficient of determination ( $R^2$ ) of the linear regression lines were compared among the lakes.

Normalised mass spectra (NMS) (13) were analysed similarly to NLS but were modified for fish-mass data (*cf.* Blanchard *et al.*, 2005; Sweeting *et al.*, 2009). All fish were allocated to  $\log_2$  FM classes from 1 (8 g) to 10 (> 2048 g). Class 1 summed fish from  $\log_2 = 2$  (4 g) and  $\log_2 = 3$  (8 g), because individuals with FM < 4 g were extremely rare (0.008%) because of the removal of all fish < 8 cm TL from the data set. Fish masses within each size class were divided by the number of nets and the span of the size class, subsequently  $\log_2(x + 1)$ -transformed and finally regressed analogously to the NLS. The slopes and  $R^2$  of the linear regression lines were calculated. Slopes of normalised size spectra (NLS and NMS here)

quantify the relative abundance of small and large fish in a sample (Shin & Cury, 2004). A steepening of the slope can be the result of a decreasing number of large fish, an increasing number of small fish or both (Pope & Knights, 1982; Bianchi *et al.*, 2000). For size–abundance relationships,  $R^2$  can be used as a measure of disturbance (*i.e.* the relative distance from steady state) in a given system (Sprules *et al.*, 1983; Choi *et al.*, 1999).

Fish mass spectra (14) were analysed using underlying Pareto type II probability density functions  $p_i(FW)$ , following the equation

$$p_i(FM) = c(K + D)^c (FM_t + D)^{-(c+1)}$$

with the cumulative distribution of probability defined as:

$$prob(FM \geq FM_t) = (K + D)^c - (FM_t + D)^c$$

where  $D$  is an additive constant,  $FM$  is the fresh mass of the individual fish and  $FM_t$  is a threshold size (Vidondo *et al.*, 1997). In other words, the term  $prob(FM \geq FM_t)$  is calculated for each fish mass as the proportion of all fish larger than or equal to itself:

$$prob(FM \geq FM_t) = \frac{N_{FM \geq FM_t}}{N_t}$$

The parameters  $c$  and  $D$  were calculated by regressing  $\log(prob(FM \geq FM_t))$  on  $FM$  using the iterative nonlinear algorithm  $\log(prob(FM \geq FM_t)) = c \log(K + D) - c \log(FM_t + D)$  (Vidondo *et al.*, 1997). Beforehand, fish masses were standardised ( $K = 1$ ) following Winiwarter & Cempel (1992):

$$FM' = \frac{FM}{FM_{\min}}$$

where the standardised masses ( $FM'$ ) were obtained by dividing the fish masses within a sample ( $FM$ ) by the minimum mass observed in that sample ( $FM_{\min}$ ).

Finally, we derived three parameters (the exponent  $c$ , the additive constant  $D$  and  $R^2$ ) from the nonlinear ordinary least square regressions. Although the ecological meaning of  $c$  and  $D$  is somewhat difficult to interpret (Quintana *et al.*, 2008) and the response of the parameters to gradients is not yet completely understood (Gamble *et al.*, 2006), they can be used to compare systems (Vidondo *et al.*, 1997). Higher values of  $c$  (and  $D$ ) are obtained in cases of more pronounced curvature in the Pareto type II distribution, whereas lower values represent more linear models. A more linear relationship indicates a higher relative abundance of smaller size classes, while a stronger curvature of the nonlinear regression line indicates a more equal distribution of the sizes with a higher relative abundance of larger size classes (Bruce *et al.*, 2005).

In general, we favoured length-related variables over mass-related ones, as fish mass is often incompletely recorded during field campaigns. In the case of the Pareto type II approach, however, FM data are required (Gamble *et al.*, 2006), as length data result in strong inaccuracies in the parameter estimates (large confidence intervals) owing to the smaller amplitude of fish length compared with FM (M. Emmrich, unpublished). To exclude strongly correlated variables from subsequent analyses, we calculated correlations between all size-related variables and selected only those with Spearman's rank correlation coefficients  $r_s < |0.6|$  (see Table 2 for detailed  $r_s$  and  $P$  values). In the case of strongly correlated variables, we favoured those for which the loss of original information in the size data was lowest.

Table 1: Characteristics and variability of 18 size-related variables from 78 German lowland lakes including variable range, mean, median, and coefficient of variation (CV). For a detailed description of the size parameters see section methods.

Size-related variable (Unit)	Code	Range		Mean	Median	CV (%)
		lower	upper			
Geometric mean length (cm)	$L_{\text{gmean}}$	9.91	16.65	12.26	12.11	10.21
Variance length data	$L_{\text{var}}$	8.63	123.43	30.29	28.30	51.03
Skewness LFD	$L_{\text{skew}}$	-0.40	1.48	0.49	0.44	71.96
Kurtosis LFD	$L_{\text{kurt}}$	-1.73	0.82	-0.92	-1.04	57.41
Number of size classes	n SC	20.00	44.00	31.56	32.00	16.83
Interquartile range length data (cm)	IQR	2.00	13.00	5.71	6.00	35.41
Maximum length (cm)	$L_{\text{max}}$	32.00	105.00	58.01	55.00	27.93
95% percentile	$L_{95}$	14.00	30.00	22.69	23.00	15.74
Proportion quality length (%)	$L_{\text{qual}}$	0.07	6.96	1.46	1.18	89.57
Fulton's condition factor assemblage	$K_a$	0.79	1.32	1.06	1.05	9.93
Size diversity	SD ( $\mu$ )	1.07	2.25	1.80	1.86	14.14
Slope normalised length spectra	S NLS	-1.46	-0.24	-0.90	-0.90	3.19
$R^2$ normalised length spectra	$R^2$ NLS	0.61	0.95	0.80	0.80	10.88
Slope normalised mass spectra	S NMS	-0.75	-0.19	-0.51	-0.51	4.48
$R^2$ normalised mass spectra	$R^2$ NMS	0.41	0.98	0.86	0.90	7.61
c Pareto type II mass spectra	c Par	0.68	8.20	1.96	1.78	45.23
D Pareto type II mass spectra	D Par	0.09	107.15	13.39	8.37	116.06
$R^2$ Pareto type II mass spectra	$R^2$ Par	0.87	0.99	0.97	0.98	2.51

LFD = length-frequency distribution

### 1.2.3 Lake variables

Non-metric multidimensional scaling (NMDS) analysis (Kruskal, 1964) was used to analyse size-related fish-assemblage differences. NMDS analysis maximises rank-order correlation between original distance measures and the distances in ordination space. The points (lakes) are moved to minimise stress, which is a measure of mismatch between both distances. Three matrices were constructed. The main matrix contained the eight weakly correlated size-related variables that were normalised by their maxima. Initially, the NMDS analysis was performed by selecting random start configuration, six dimensions, Euclidean distance and 200 iterations. Monte Carlo simulation (50 runs with real and randomised data) was included to check whether a similar final stress value was obtained by chance. The optimum dimensionality was derived from the scree plot of stress versus dimensionality. The configuration of the optimal dimensionality was selected as the new starting configuration for the final run without a further change in dimensionality. Additionally, Spearman's correlations of size-related variables with the significant axes were calculated.

In two comatrices, a total of 17 abiotic and biotic descriptors of the 78 lakes and their fish assemblages were summarised. The first comatrix included nine abiotic variables, divided into (1) variables describing lake morphometry ( $n = 5$  variables), (2) lake productivity variables ( $n = 2$ ) and (3) variables characterising lake-use intensity ( $n = 2$ ). In detail, the morphometric variables (1) consisted of (variable range in brackets) lake area (50–11,300 ha), maximum depth (1.0–72.3 m), mean depth (0.65–28.6 m), shore length (3.0–123.7 km) and catchment area (190–750,000 ha).

The productivity variables (2) encompassed total phosphorus concentration (13.0–330.0  $\mu\text{g L}^{-1}$ ) and chlorophyll *a* concentration (Chl *a*) (1.5–287.7  $\mu\text{g L}^{-1}$ ), both based on arithmetic averages from samplings taken during the growing season between May and September. Morphometry and productivity variables were  $\log_{10}$ - or  $\log_{10}(x + 1)$ -transformed to ensure a linear relationship and to stabilise their variances. Lake-use intensity (3) (human-use variables, anthropogenic shoreline modifications and fishing activity) was initially assessed on a ranked scale (all variables except fishing activity): category 1 = no impact; category 2 = minor; category 3 = moderate; category 4 = heavy impact. The human-use variables included the frequency of commercial ship passages, the number of recreational boats with and without engines, bathing and fishing activities. Anthropogenic shoreline modifications included bathing places, footbridges/marinas, sheet piles, woody erosion control structures (fascines) and rip-rap habitats. Fishing activity was classified as 1 = no fisheries; 2 = recreational fisheries; 3 = commercial fisheries; 4 = both recreational and commercial fisheries. For the conversion of categorical variables into continuous synthetic ones, we

used multiple correspondence analysis (MCA; Tenenhaus & Young, 1985; Greenacre & Blasius, 2006) by building an indicator matrix with a binary coding of the 10 lake-use variables with the four categories of each factor. The principal coordinates for each lake of the first two MCA axes were considered as synthetic lake-use variables (axis 1 + 2 lake use).

To a second comatrix, we added eight biotic variables (4) that describe the taxonomic or functional composition of the fish assemblages in lakes. Three variables reflected taxonomic composition, namely number of species, proportion of cyprinids and proportion of percids (Table 3). Three further variables reflected the predator-prey relationships in the lakes. According to Mehner (2010), pike (*Esox lucius* L.), zander [*Sander lucioperca* (L.)], burbot (*Lota lota* L.), asp [*Aspius aspius* (L.)] and European catfish (*Silurus glanis* L.) were classified as predators (obligatory piscivores). Perch were divided into predatory (TL  $\geq$  15 cm) and non-predatory (TL < 15 cm) fish (*cf.* Persson *et al.*, 1992). All other fish were considered as potential prey. We calculated the numerical proportion (%) and biomass proportion (%) of predators in each lake. Predator-prey length ratio (PPLR) was calculated as the ratio between mean predator length and mean prey length.

Finally, two further variables indicated species diversity in the lakes. In correspondence to the size diversity index mentioned earlier (Quintana *et al.*, 2008), we calculated the taxonomic Shannon index ( $H'$ ) by

$$H' = -\sum_{i=1}^n p_i \log_2 p_i$$

and evenness ( $J$ ) as

$$J = \frac{H'}{\log_2 s}$$

with  $s$  representing the number of species.

Taxonomic and functional variables of the fish assemblages were normalised to their maxima, and proportion data were arc-sine square-root-transformed (Sokal & Rohlf, 1995). After ordination of the main matrix (size-related variables) by NMDS analysis, Spearman's rank correlations between the lake's scores per dominant axis and the lake-specific variables included in the two comatrices were calculated. NMDS analysis was performed by PC-Ord for Windows, version 4 (McCune & Mefford, 1999; MJM Software Design, Gleneden Beach, OR, U.S.A.). MCA was computed in R (version 2.7.0; R Development Core Team, 2008) using the *ca* package (version 0.2.1, 2007; Nenadić & Greenacre, 2007).

Table 2: Correlation matrix of the 18 size-related variables. The white matrix contains Spearman's  $r$  values; the grey matrix contains the corresponding  $P$ -values. Weakly correlated variables ( $r_s < |0.60|$ ) that were exposed to the non-metric multidimensional scaling ordination are indicated in bold. For the full names of the size-related variables see Table 1.

Variable	L <sub>gmean</sub>	L <sub>var</sub>	L <sub>skew</sub>	L <sub>kurt</sub>	n SC	IQR	L <sub>max</sub>	L <sub>95</sub>	L <sub>qual</sub>	K <sub>a</sub>	SD ( $\mu$ )	S NLS	<b>R<sup>2</sup> NLS</b>	S NMS	<b>R<sup>2</sup> NMS</b>	c Par	D Par	<b>R<sup>2</sup> Par</b>
L <sub>gmean</sub>	---	0.564	-0.579	-0.590	0.311	0,733	0.157	0.559	0.560	0.197	0.688	0.246	0.655	0.415	-0.403	0.322	0.552	0.078
L <sub>var</sub>	<b>&lt;0.001</b>	---	-0.696	-0.369	0.673	0,668	0.359	0.853	0.821	0.081	0.825	0.289	0.418	0.460	0.060	0.027	0.442	-0.124
L <sub>skew</sub>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	---	0.718	-0.567	-0.708	-0.137	-0.812	-0.629	-0.234	-0.751	-0.053	-0.674	-0.184	-0.064	-0.469	-0.787	0.086
L <sub>kurt</sub>	<b>&lt;0.001</b>	0.001	<b>&lt;0.001</b>	---	-0.184	-0.642	-0.183	-0.421	-0.214	-0.193	-0.655	-0.218	-0.719	-0.231	0.102	-0.472	-0.616	-0.174
<b>n SC</b>	0.006	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.106	---	0,370	0.387	0.579	0.680	-0.028	0.510	-0.123	0.355	0.007	0.345	-0.145	0.236	0.049
IQR	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.001	---	0.208	0.601	0.564	0.233	0.820	0.233	0.631	0.375	-0.188	0.408	0.656	0.026
<b>L<sub>max</sub></b>	0.170	0.001	0.233	0.108	<b>&lt;0.001</b>	0.068	---	0.097	0.172	-0.228	0.261	0.203	0.002	0.232	0.049	-0.362	-0.180	0.224
L <sub>95</sub>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.399	---	0.806	0.188	0.764	0.195	0.471	0.364	0.093	0.233	0.593	-0.266
L <sub>qual</sub>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.060	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.133	<b>&lt;0.001</b>	---	0.047	0.707	0.243	0.419	0.420	0.059	-0.044	0.378	-0.149
<b>K<sub>a</sub></b>	0.084	0.479	0.040	0.090	0.804	0.040	0.045	0.099	0.680	---	0.152	-0.265	0.280	-0.117	-0.437	0.545	0.436	-0.088
<b>SD (<math>\mu</math>)</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.021	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.185	---	0.296	0.546	0.444	-0.003	0.188	0.530	0.082
<b>S NLS</b>	0.030	0.010	0.646	0.056	0.285	0.040	0.074	0.088	0.032	0.019	0.009	---	0.178	0.914	-0.007	-0.184	-0.034	0.004
<b>R<sup>2</sup> NLS</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.001	<b>&lt;0.001</b>	0.989	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.013	<b>&lt;0.001</b>	0.102	---	-0.067	-0.150	0.481	0.656	0.178
S NMS	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.108	0.042	0.949	0.001	0.041	0.001	<b>&lt;0.001</b>	0.306	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.561	---	-0.167	-0.108	0.099	-0.054
<b>R<sup>2</sup> NMS</b>	<b>&lt;0.001</b>	0.600	0.578	0.372	0.002	0.099	0.668	0.419	0.608	<b>&lt;0.001</b>	0.981	0.953	0.190	0.143	---	-0.328	-0.209	-0.025
<b>c Par</b>	0.004	0.818	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.205	<b>&lt;0.001</b>	0.001	0.040	0.702	<b>&lt;0.001</b>	0.099	0.107	<b>&lt;0.001</b>	0.346	0.003	---	0.830	-0.086
D Par	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.038	<b>&lt;0.001</b>	0.114	<b>&lt;0.001</b>	0.001	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.768	<b>&lt;0.001</b>	0.389	0.067	<b>&lt;0.001</b>	---	-0.157
<b>R<sup>2</sup> Par</b>	0.499	0.280	0.451	0.127	0.669	0.823	0.049	0.019	0.193	0.446	0.474	0.972	0.119	0.641	0.828	0.455	0.170	---

Table 3: Pearson's correlation coefficients between variables of lake morphometry, lake productivity, lake-use intensity, and taxonomic and functional descriptors of fish communities and the lake scores of the three dominant axes (predicted variance in brackets) obtained from the non-metric multidimensional scaling (NMS). NMS was based on seven size-related variables derived from fish communities sampled in 78 lowland lakes in northern Germany. Significantly correlated variables ( $r > |0.30|$ ,  $P \leq 0.006$ ) are indicated in bold.

Variable	Unit	Axis 1 (15.6%)	Axis 2 (40.8%)	Axis 3 (40.4%)
<b>Lake morphometry</b>				
Area	ha	<b>-0.324</b>	-0.060	-0.090
Shore length	km	-0.214	-0.209	0.045
Maximum depth	m	0.250	<b>-0.582</b>	<b>0.465</b>
Mean depth	m	<b>0.319</b>	<b>-0.515</b>	<b>0.417</b>
Catchment area	ha	<b>-0.410</b>	0.163	-0.165
<b>Lake productivity</b>				
Total phosphorus	$\mu\text{g L}^{-1}$	-0.275	<b>0.349</b>	<b>-0.472</b>
Chlorophyll <i>a</i>	$\mu\text{g L}^{-1}$	-0.253	<b>0.442</b>	<b>-0.474</b>
<b>Lake-use intensity (MCA scores)</b>				
Axis 1 lake use		0.283	-0.010	0.103
Axis 2 lake use		-0.103	0.101	-0.183
<b>Fish assemblage descriptors</b>				
Proportion cyprinids	%	-0.130	0.297	-0.143
Proportion percids	%	0.031	-0.165	-0.021
Proportion predators (biomass)	%	0.125	-0.295	<b>0.357</b>
Proportion predators (abundance)	%	0.042	<b>-0.358</b>	0.006
Predator-prey length ratio		0.173	0.070	<b>0.418</b>
Number of species		<b>-0.401</b>	-0.138	0.298
Shannon diversity		-0.167	-0.048	-0.092
Shannon evenness		0.050	0.003	-0.230

## 1.3 Results

### 1.3.1 Size-related variables

In total, size data of 132,665 fish  $\geq 8$  cm total length caught by multi-mesh gillnets in 78 north German lowland lakes were included in this study. Catch size ranged from 407 to 4248 individuals per lake (mean 1645 individuals).

Differences in range and coefficient of variation (CV) were substantial between the 18 size-related variables tested (Table 1). The highest variability was found in the additive constant  $D$  of the Pareto type II mass spectra, the skewness of the length–frequency distributions and in the proportion of fish above quality length. Variability in the slopes and  $R^2$  of the normalised size spectra was similar with lower variability in the slopes in comparison with their corresponding  $R^2$  values. High values of size diversity were obtained in cases of equally distributed fish lengths (Fig. 2a) or if the catch consists of many different fish lengths (Fig. 2b). For fish-mass data, the relative proportion of small and medium-sized fish has the strongest influence on the exponent  $c$  of the Pareto type II mass spectra (Figs 2c, d & 8).

Many size-related variables were strongly correlated (Table 2). To avoid redundant information, we selected for further analyses only those variables with Spearman's  $r < |0.6|$  in correlations or those that contained most information from the original data. Accordingly, size diversity (SD) was favoured over  $L_{\text{gmean}}$  (correlation with SD:  $r_s = 0.69$ ),  $L_{\text{var}}$  ( $r_s = 0.83$ ),  $L_{\text{skew}}$  ( $r_s = -0.75$ ),  $L_{\text{kurt}}$  ( $r_s = -0.66$ ), IQR ( $r_s = 0.82$ ),  $L_{95}$  ( $r_s = 0.76$ ) and  $L_{\text{qual}}$  ( $r_s = 0.71$ ) because size diversity contains information on the amplitude of the size range and the relative distribution of sizes along the size range (Quintana *et al.*, 2008). Furthermore, strong correlations existed between those variables that we decided to exclude in favour of size diversity (Table 2). The number of size classes ( $n_{\text{SC}}$ ) was only strong correlated with  $L_{\text{qual}}$ , which was already excluded. Maximum length and the condition factor of the fish assemblage were not correlated with any other size-related variable ( $r_s < 0.55$ ).

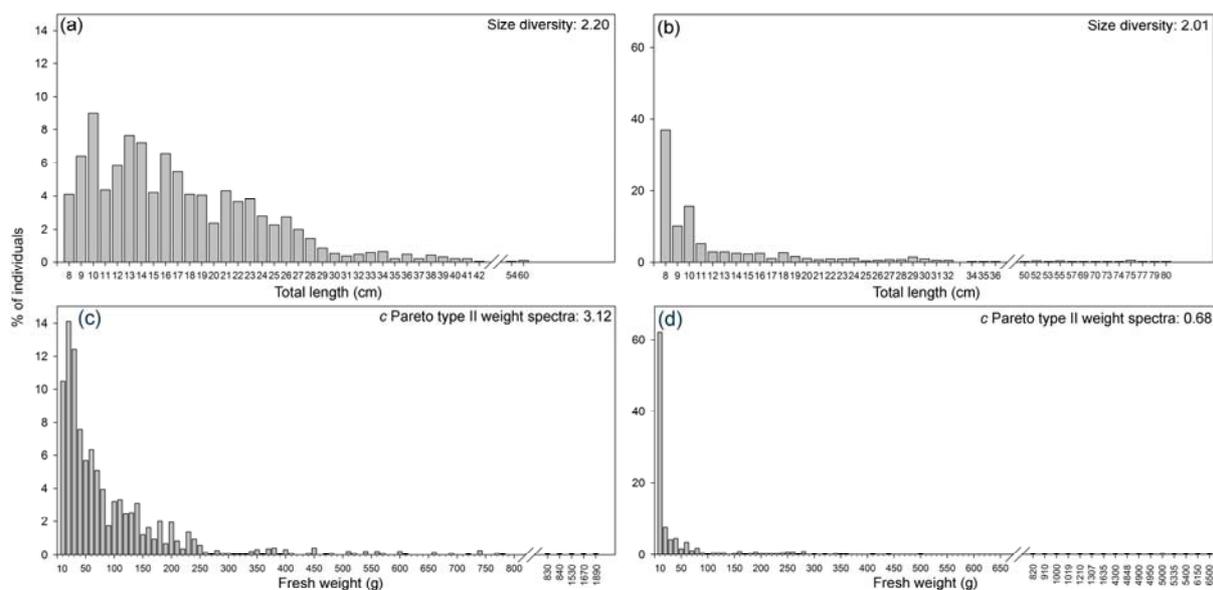


Figure 2: Examples of fish-length distributions from two lakes with similar values of size diversity (a; b) and their corresponding mass distributions with substantial differences in their exponents  $c$  of the Pareto type II mass distributions (c; d).

In the case of normalised length and mass-spectra slopes ( $r_s = 0.91$ ), we selected the slopes of the normalised length spectra over the ones of the mass spectra because parameters of the Pareto type II mass spectra also described the mass distribution. No classification or transformation of the mass data is necessary in the Pareto approach (Blanco, *et al.*, 1994; Vidondo *et al.*, 1997). The mass of each individual fish was included in the calculations resulting in a higher mean  $R^2$  value (0.97) in comparison with the mean  $R^2$  of the normalised mass spectra (0.86). We selected only the exponent  $c$  of the Pareto type II mass spectra, because the additive constant  $D$  was highly redundant (correlations with  $c$ :  $r_s = 0.83$ ; IQR:  $r_s = 0.66$ ).

Ultimately, eight size-related variables were included in the main matrix and exposed to a NMDS analysis: number of size classes, maximum length, Fulton's condition factor, parameter  $c$  and  $R^2$  of the Pareto type II mass spectra, slope and  $R^2$  of the normalised length spectra and size diversity.

The NMDS analysis on eight size-related variables resulted in a three-dimensional solution (Fig. 3; final stress = 6.84, final instability = 0.00001 at 164 iterations).

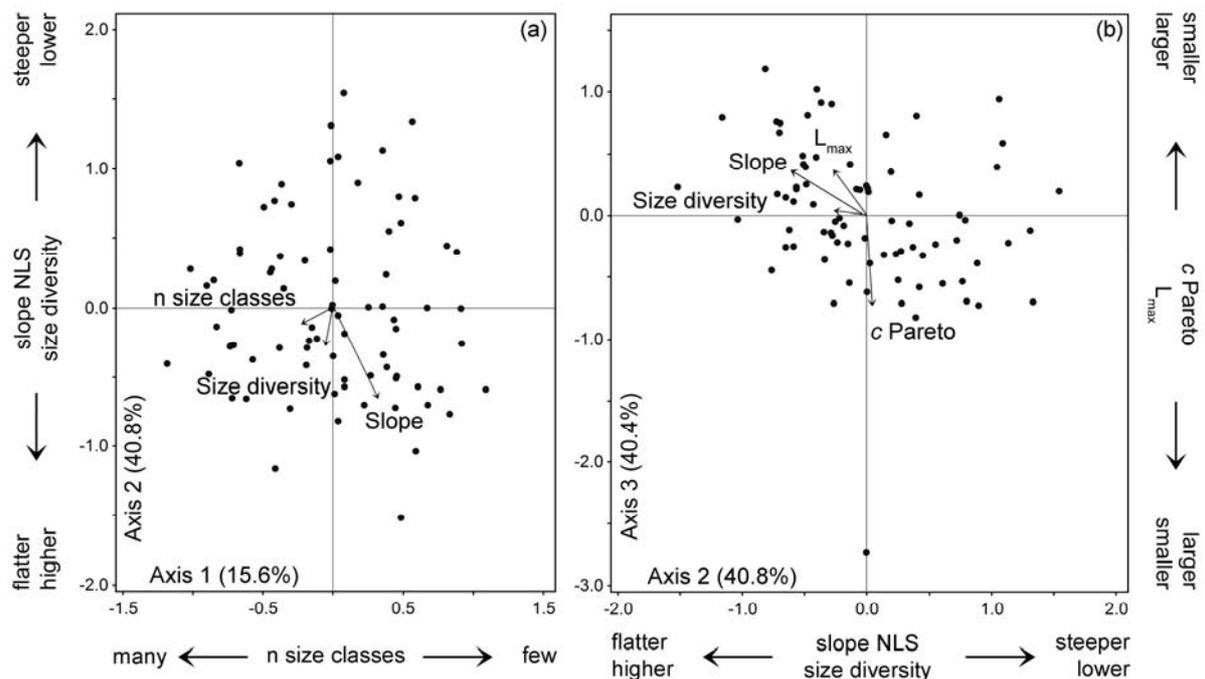


Figure 3: Plots obtained from non-metric multidimensional scaling analysis based on eight size-related variables derived from fish assemblages of 78 lakes sampled with multi-mesh gillnets. Two plots of the three-dimensional solution (96.8% predicted variance) are shown (predicted variance in brackets): Axis 1 vs. axis 2 (a) and axis 2 vs. axis 3 (b), both including correlation vectors of significant size-related variables with Spearman's  $r > |0.60|$ . For better illustration, vectors were enlarged to 400%. Interpretation of the axes scores are given on the horizontal and vertical axes. The slope was calculated from normalised length spectra (NLS), the exponent  $c$  was calculated from Pareto type II mass spectra;  $L_{max}$  = maximum length

The three axes accounted for 96.8% of the variation. The first axis (15.6% predicted variance) was negatively correlated with the number of size classes (Spearman's  $r = 0.79$ ). The second axis (40.8%) was negatively correlated with the slope of the normalised length spectra ( $r_s = 0.88$ ) and size diversity ( $r_s = -0.64$ ). The third axis (40.4%) was negatively correlated with the exponent  $c$  of the Pareto type II mass spectra ( $r_s = -0.65$ ) and positively correlated with maximum length ( $r_s = 0.73$ ). All other size-related variables were less strongly correlated with the NMDS axes ( $r_s < |0.53|$ ).

### 1.3.2 Ordination of lake-use intensity variables

The first two axes of the MCA explained 32.7% of the predicted variance (axis 1: 20.6% and axis 2: 12.1%). Axis 1 was strongly correlated with the frequency of footbridges/marinas (Spearman's  $r = 0.85$ ), the frequency of recreational boats with and without engines ( $r_s = 0.74$  and  $r_s = 0.76$ ), bathing places ( $r_s = 0.74$ ) and bathing activities ( $r_s = 0.73$ ). The second axis was correlated with the frequency of footbridges/marinas ( $r_s = 0.47$ ), types of fishing activities ( $r_s = 0.45$ ), frequency of fascines ( $r_s = 0.41$ ) and sheet piles ( $r_s = 0.42$ ). The scores of lakes on these two axes were included in the comatrix of abiotic lake descriptors for the NMDS analysis.

### 1.3.3 Response of size-related variables along gradients

Fifteen variables from three groups of lake and fish assemblage descriptors were strongly correlated (Spearman's  $r > |0.30|$ ,  $P \leq 0.004$ ) with the axes of the NMDS analysis on size-related variables (Table 3; Figs 4–6), thus reflecting correspondence of size related variables to lake morphometry, lake productivity and functional fish-assemblage composition. The number of size classes increased with increasing lake area and decreased with increasing mean depth (Fig. 4a, b). Furthermore, more size classes were observed in lakes with larger catchment areas and lakes with more species caught in the gillnets (Fig. 4c, d). The slopes of the normalised length spectra were flatter (*i.e.* less negative = fewer small, more large fish or both), and size diversity was higher in deep lakes (Fig. 5a, b) and in lakes with lower nutrient concentrations (Fig. 5c, d). Additionally, flatter slopes and higher size diversity were observed where the numerical abundance of piscivorous fish was high (Fig. 5e).

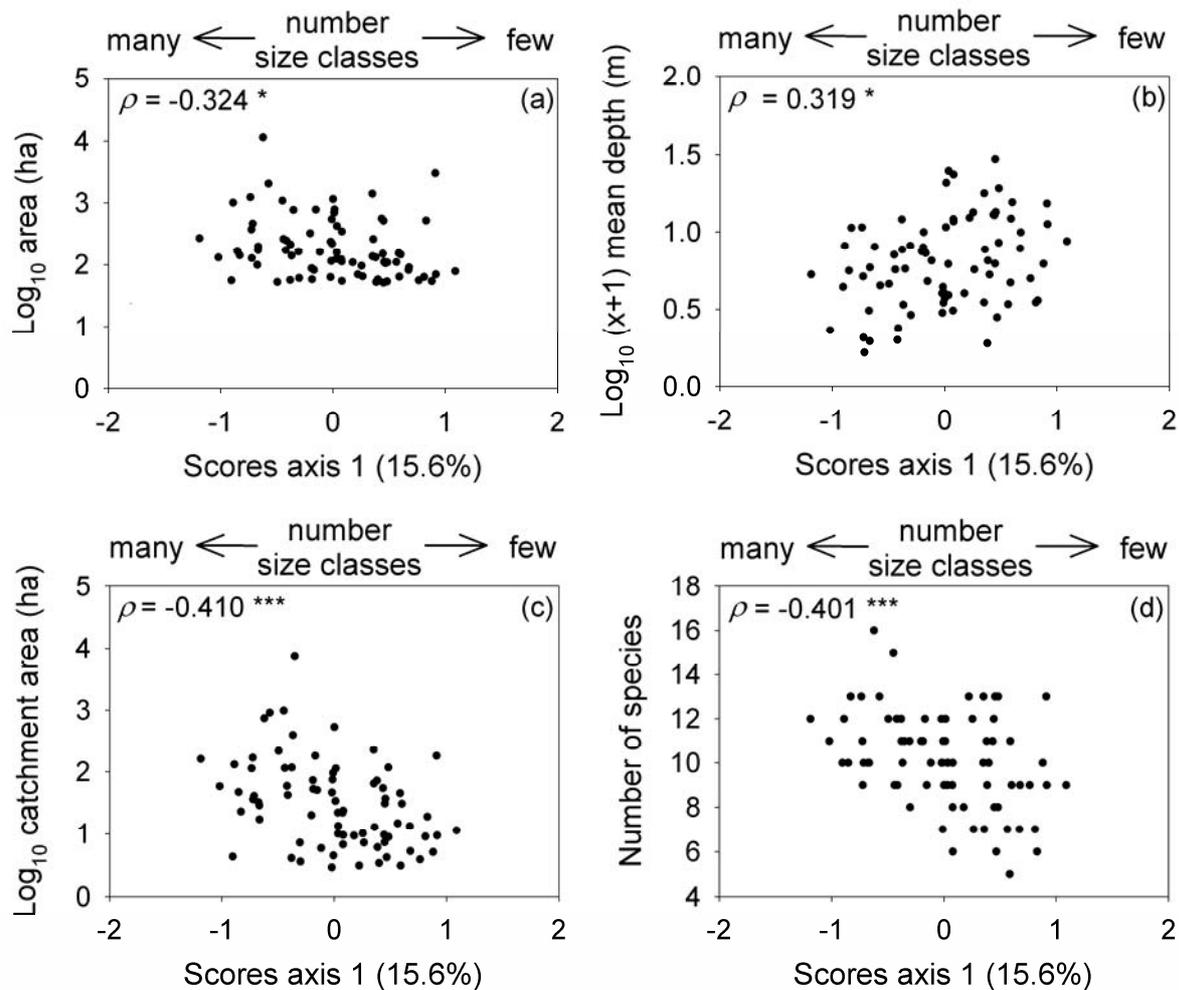


Figure 4: Scatter plots of correlated (Spearman's  $r > |0.30|$ ) variables with the scores of axis 1 of the non-metric multidimensional scaling analysis based on eight size-related variables. Interpretation of the axes scores are given on the top horizontal axes. Spearman rank correlation coefficients ( $r_s$ ) and level of significance are given in addition (\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ).

The exponent  $c$  of the Pareto type II mass spectra was larger (*i.e.* a higher relative proportion of medium-sized fish), and the maximum observed fish length in the gillnets was smaller in shallow (Figs 6a, b & 8a; Table 4a) and nutrient-rich lakes (Figs 6c, d & 8a; Table 4a). A higher proportion of predator biomass and higher predator-prey length ratios (influenced by a strong increase in arithmetic mean predator lengths but only a slight decrease in mean prey lengths (Fig. 7) were associated with smaller exponents and larger maximum lengths (Figs 6e, f & 8d; Table 4d).

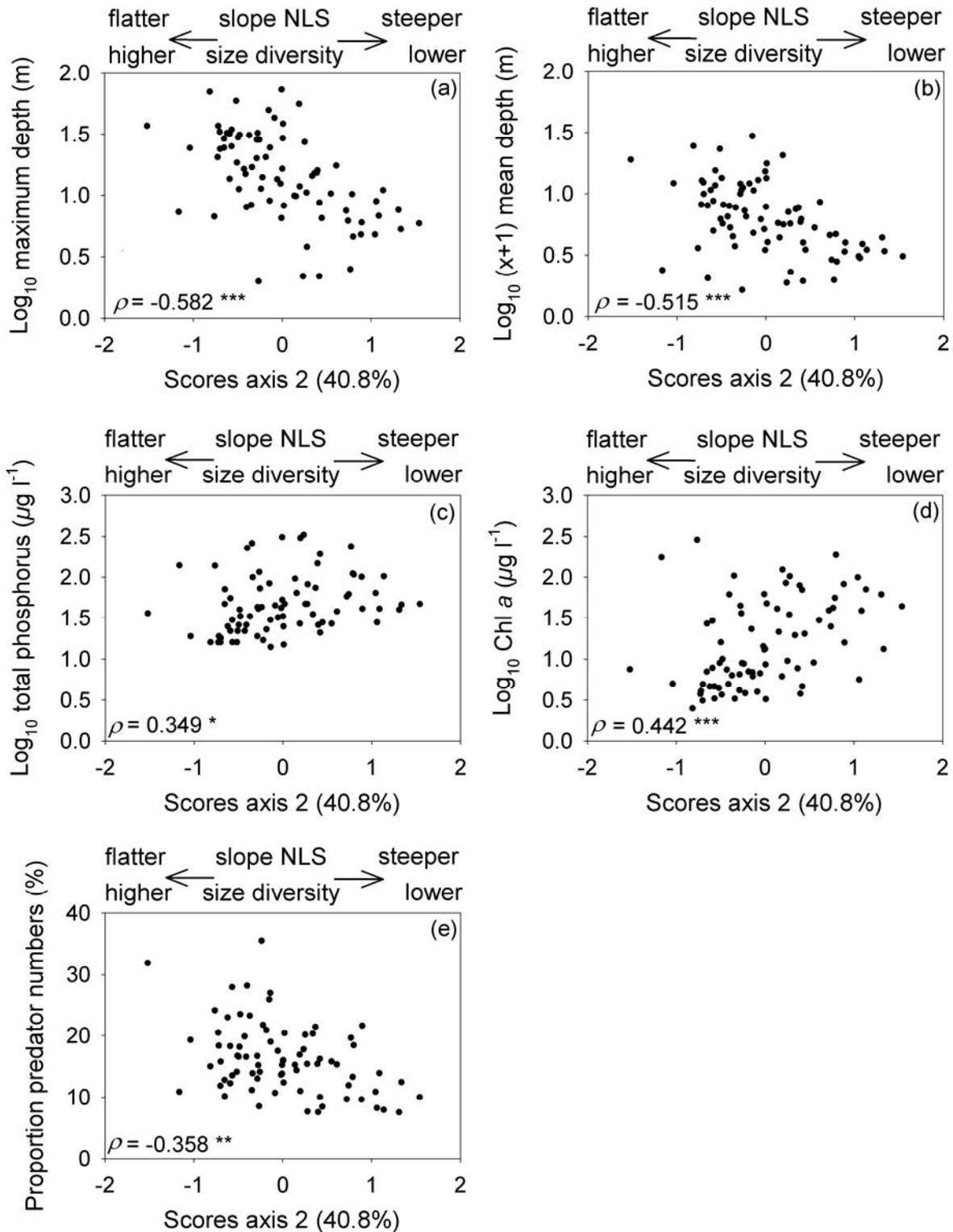


Figure 5: Scatter plots of correlated (Spearman's  $r > |0.30|$ ) variables with the scores of axis 2 of the non-metric multidimensional scaling analysis based on eight size-related variables. Interpretation of the axes scores are given on the top horizontal axes. Spearman rank correlation coefficients ( $r_s$ ) and level of significance are given in addition (\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ). slope NLS: slope of normalised length spectra

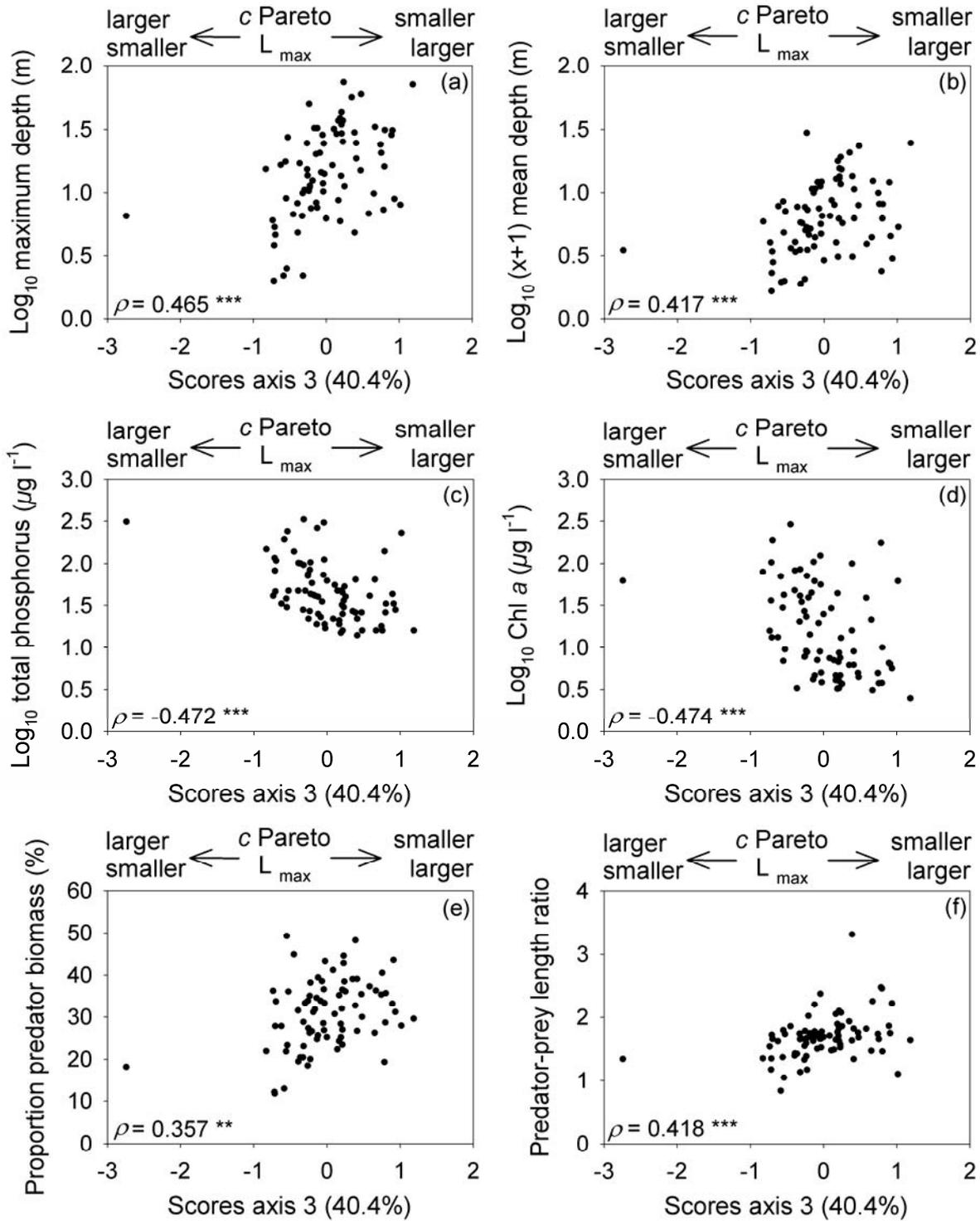


Figure 6: Scatter plots of correlated (Spearman's  $r > |0.30|$ ) variables with the scores of axis 3 of the non-metric multidimensional scaling analysis based on eight size-related variables. Interpretation of the axes scores are given on the top horizontal axes. Spearman rank correlation coefficients ( $r_s$ ) and level of significance are given in addition (\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ ).  
 c Pareto: exponent of the Pareto type II mass spectra,  $L_{\text{max}}$  = maximum length

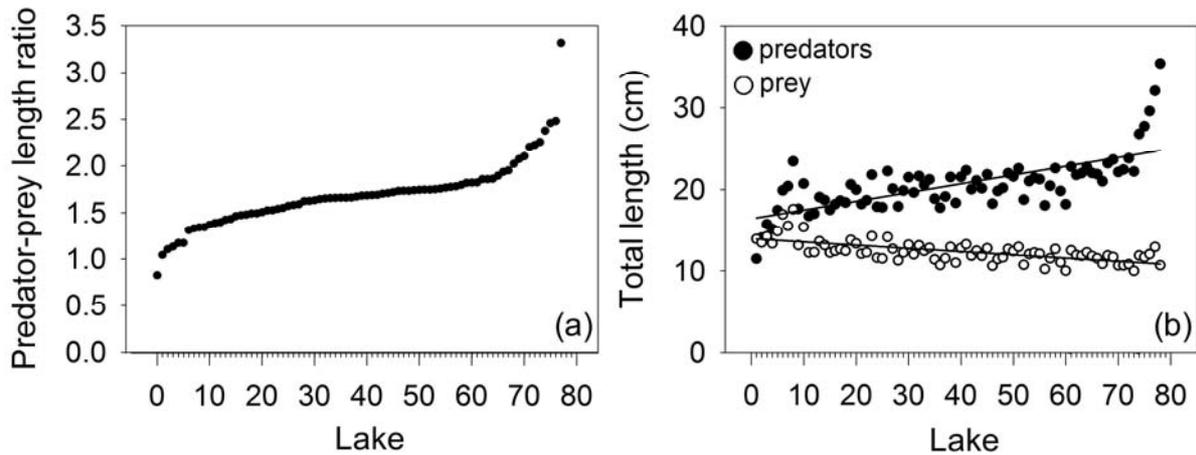


Figure 7: Predator-prey length ratio of the 78 German lakes (a) calculated as the ratio between mean predator length and mean prey length (b).

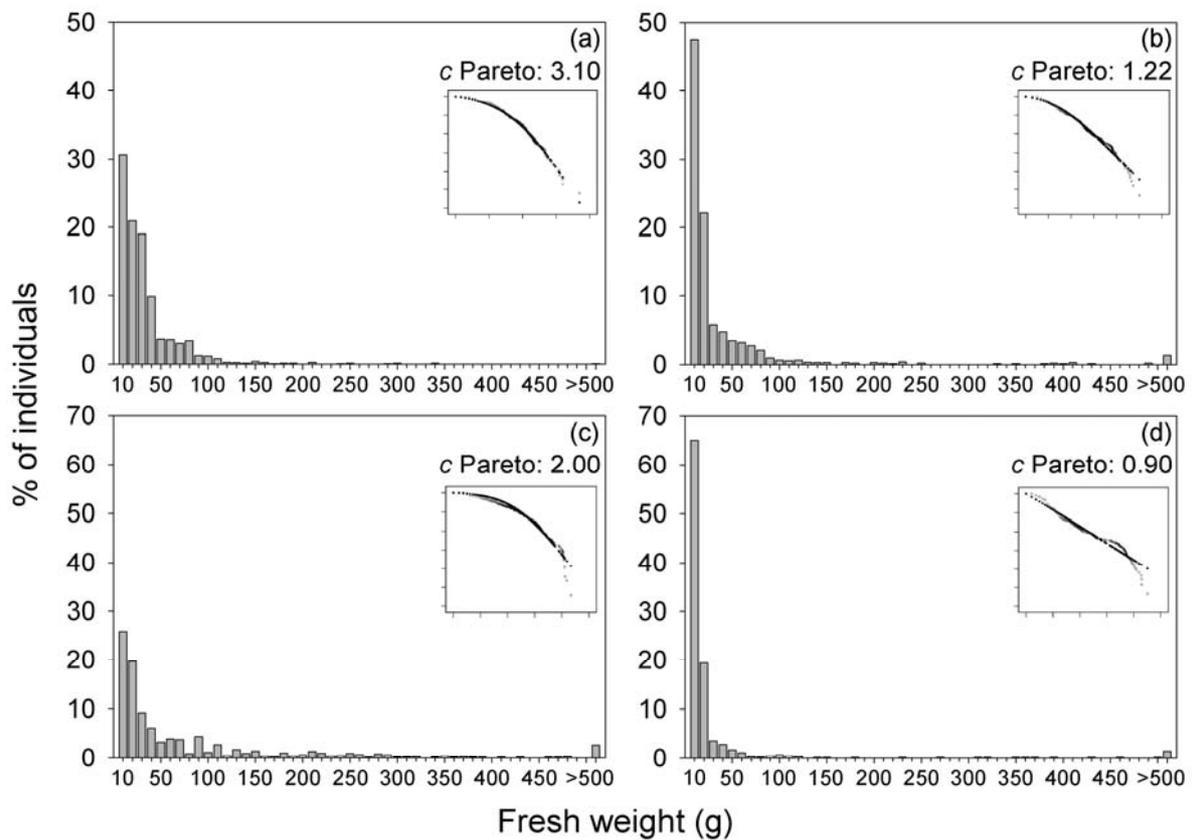


Figure 8: Four examples of mass distributions and exponents  $c$  of their corresponding Pareto type II mass spectra (insets) of lake fish assemblages sampled with multi-mesh gillnets. The lakes differed in those descriptors that were significantly correlated with the lake scores derived from the non-metric multidimensional scaling analysis (*cf.* Fig. 6; Table 4):

(a): shallow, nutrient-rich lake; (b): deep, less nutrient-rich lake; (c): low predator biomass, low predator-prey length ratio (PPLR); (d): high predator biomass, high PPLR.

Table 3: Pearson's correlation coefficients between variables of lake morphometry, lake productivity, lake-use intensity, and taxonomic and functional descriptors of fish communities and the lake scores of the three dominant axes (predicted variance in brackets) obtained from the non-metric multidimensional scaling (NMS). NMS was based on seven size-related variables derived from fish communities sampled in 78 lowland lakes in northern Germany. Significantly correlated variables ( $r > |0.30|$ ,  $P \leq 0.006$ ) are indicated in bold.

Variable	Unit	Axis 1 (9.8%)	Axis 2 (43.2%)	Axis 3 (40.6%)
<b>Lake morphometry</b>				
Area	ha	-0.174	-0.116	-0.116
Shore length	km	-0.077	-0.207	0.038
Maximum depth	m	<b>0.311</b>	<b>-0.510</b>	<b>0.517</b>
Mean depth	m	<b>0.383</b>	<b>-0.440</b>	<b>0.456</b>
Catchment area	ha	-0.243	0.121	-0.204
<b>Lake productivity</b>				
Total phosphorus	$\mu\text{g l}^{-1}$	-0.162	0.232	<b>-0.505</b>
Chlorophyll a	$\mu\text{g l}^{-1}$	-0.150	<b>0.358</b>	<b>-0.447</b>
<b>Lake-use intensity (NMS scores)</b>				
Axis 1 lake use		-0.086	0.233	<b>-0.310</b>
Axis 2 lake use		0.068	0.088	0.063
<b>Fish community descriptors</b>				
Proportion cyprinid hybrids	%	-0.262	0.113	-0.295
Proportion cyprinids	%	-0.187	0.293	-0.082
Proportion percids	%	0.024	-0.209	-0.055
Proportion predators (biomass)	%	0.096	-0.151	<b>0.303</b>
Proportion predators (abundance)	%	-0.005	<b>-0.385</b>	-0.077
Predator-prey length ratio		0.138	0.240	<b>0.539</b>
Number of species		-0.270	-0.178	0.244
Shannon index		-0.048	-0.085	-0.079
Shannon evenness		0.086	-0.007	-0.230

Table 4: Descriptors for of lake morphometry, lake productivity and functional fish assemblage composition of the four example lakes whose fish-weight distributions are shown in Fig. 8. Significant differences in lake descriptors are indicated in bold. (a): shallow, nutrient-rich lake; (b): deep, less nutrient-rich lake; (c): low predator biomass, low predator-prey length ratio (PPLR); (d): high predator biomass, high PPLR;

Lake	(a)	(b)	(c)	(d)
Maximum depth (m)	<b>5.0</b>	<b>19.1</b>	6.5	6.1
Mean depth (m)	<b>3.0</b>	<b>8.9</b>	1.3	2.1
Total phosphorus ( $\mu\text{g l}^{-1}$ )	<b>40.0</b>	<b>18.0</b>	81.0	63.0
Chlorophyll a ( $\mu\text{g l}^{-1}$ )	<b>15.9</b>	<b>3.2</b>	102.0	98.4
Predator biomass (%)	36.3	34.6	<b>11.8</b>	<b>48.2</b>
PPLR	1.54	1.63	<b>1.3</b>	<b>3.3</b>

## 1.4 Discussion

The comparison of 18 size-related variables derived from multi-mesh gillnet catches from 78 German lowland lakes showed substantial variation, even within a data set limited in geographical range. Many of the variables were strongly correlated. An ordination of the lakes, based on eight weakly correlated size descriptors with slope of normalised length spectra, size diversity, exponent  $c$  of Pareto type II mass spectra, maximum length and number of size classes as the most important ones, was correlated with descriptors of lake morphometry, lake productivity and functional fish-assemblage composition. This suggests that size spectra can be a useful tool for identifying systematic variation in fish assemblages along environmental gradients.

Lake area was positively correlated with the number of size classes, indicating a wider range of fish sizes with more large sizes in large lakes. Similarly, Sumari (1971) found more size classes of perch in larger ponds. Our results are also in accordance with empirical studies (Holmgren & Appelberg, 2000; Wilde & Pope, 2004) supporting the theory that larger lakes are inhabited by larger fish. As for European lakes in general (Nöges, 2009), lake area was positively correlated with catchment area in our study (Pearson's  $r$  for  $\log_{10}$ -transformed lake and catchment area: 0.66;  $P < 0.001$ ). Additionally, species richness in the gillnets was correlated with lake area ( $r = 0.36$ ;  $P = 0.001$ ; cf. Eckmann, 1995). We conclude that the correlation of catchment area and species richness with axis 1 of the NMDS analysis was a consequence of their positive correlations with lake size. As we included only lakes  $> 50$  ha in our study, we cannot preclude the possibility that lake area becomes more important for other size-related variables if smaller lakes are considered as well.

In addition to lake area, we showed also that lake depth influenced the size structure of fish assemblages, because depth-related variables were correlated with all significant size variables. In lakes of greater mean depth, fewer size classes were found, which contradicts our initial assumption that deeper lakes would be associated with a higher diversity of fish size. Deeper lakes are often associated with higher habitat heterogeneity, thus reducing competition and providing habitat for more different age (size) classes (Persson, 1983). The opposite results from the different studies suggest that the correspondence between lake depth and number of size classes has to be reanalysed in a larger data set.

The second axis of the NMDS analysis was negatively correlated with the slope of the normalised length spectra and size diversity. Both size metrics showed similar responses to variables of lake depth, nutrient concentrations and predator abundance, because the slope of normalised length spectra and size diversity identify patterns in the relative proportion of small and large fish in the catches. Flatter (less negative) slopes indicate fewer small fish, more large fish or both (Pope & Knights, 1982; Bianchi *et al.*, 2000). Higher values of size

diversity are obtained if the relative proportion of large fish is higher, which is supported by the strong correlation of size diversity with  $L_{gmean}$ ,  $L_{var}$ , IQR and the strong correlations with variables directly corresponding to large fish in the catches ( $L_{qual}$  and  $L_{95}$ ).

Deep lakes, and lakes with lower nutrient concentrations and a higher numerical proportion of predators, have flatter slopes and higher size diversity. This suggests either a lower survival rate of small fish or a higher abundance of large fish in those lakes. Although a high abundance of predators is characteristic of deep, mesotrophic lakes (Persson *et al.*, 1991; Jeppesen *et al.*, 2000), Mehner (2010) recently failed to demonstrate a negative relationship between the abundance of piscivorous fish and their potential prey in German lakes. Accordingly, we conclude that flatter slopes and higher values of size diversity are primarily caused by a high proportion of large fish rather than indicating high mortality rates of small fish because of predation. However, the single effects of predators on the size structure of fish assemblages were somewhat difficult to disentangle because we included both predatory and prey fish in calculations of the size-related variables. Therefore, the observed patterns reflect in part the positive correlation between the abundance of large fish (42% of all fish > 30 cm TL were classified as predators) and flatter slopes and higher size-diversity values. Nevertheless, we suggest that predation is one of the major forces shaping the size structure of lake fish assemblages (Jackson, Peres-Neto & Olden, 2001; Kerr & Dickie, 2001) even if no negative abundance relationship can be found (Mehner, 2010). Further studies are needed to analyse in detail the impact of piscivorous fish (*i.e.* the abundance and size of predators) on the size structure of the total fish assemblage and their potential prey.

The exponent  $c$  of the Pareto type II mass spectra and maximum length were correlated with six lake and fish-assemblage variables. Exponents were largest if the size range of the gillnet catch was narrow and characterised by a more even distribution of fish mass, with a greater relative abundance of medium-sized fish. According to axis 3 of the NMDS analysis, we found smaller maximum fish lengths and a higher relative proportion of medium-sized fish in shallow, nutrient-rich lakes. The correlation between lake morphometry and nutrient status is characteristic of European lakes (Nöges, 2009), because rapid nutrient recycling in the extensive littoral zone favours higher productivity (Ryder, 1965; Hanson & Leggett, 1982). The relative increase in medium-sized fish contradicts in part the results of Jeppesen *et al.* (2000), who found a decline in mean body mass of the dominant fish species perch, roach [*Rutilus rutilus* (L.)] and bream [*Abramis brama* (L.)] in Danish lakes along an increasing nutrient gradient. However, the Danish lakes were on average shallower (mean depth 3.4 m) than the lakes in our study (6.9 m), suggesting weaker interaction strengths in German lakes. At a given nutrient concentration, volumetric fish densities and productivity decline with increasing lake depth (Ryder, 1965; Jeppesen *et al.*, 1997), thus reducing the interaction

strength in deeper lakes (Jeppesen *et al.*, 1997) as a consequence of reduced habitat coupling, which is primarily mediated by fish (Jackson *et al.*, 2001; Schindler & Scheuerell, 2002; Dolson *et al.*, 2009). Furthermore, the exponent  $c$  was smaller where predator biomass and predator-prey length ratios were large. An increase in the predator-prey length ratio was primarily caused by an increase in predator length. Consequently, a few large predators with a high biomass have a limited regulatory impact on the many small fish.

Maximum fish length increased with increasing lake depth and decreasing nutrient concentration. It has been argued that sample size and sampling effort determine the maximum length of fish caught (Shin *et al.*, 2005). We found no correlation between maximum fish length and the number of fish caught or the number of nets set. Nevertheless, it has been shown that very large fish are not adequately represented in multi-mesh gillnets if only the standard effort is applied (Holmgren & Appelberg, 2000; Pope *et al.*, 2005), which makes a coherent interpretation of this size metric more difficult. However, although large fish in lakes have larger home ranges (Minns, 1995), the probability of catching them in large, deep lakes will certainly not increase, because the very large pelagic zone is only poorly sampled by gillnets (Deceliere-Verges & Guillard, 2008). Consequently, we can assume that deeper lakes are inhabited by more fish of a maximum size. Wilde & Pope (2004) argued that large fish are less vulnerable to exploitation in large lakes, and it has been shown that fishing pressure can act as a major force shaping the size structure of lake fish assemblages (McDonald & Hershey, 1989; Lewin *et al.*, 2006). We did not detect any significant effect of fishing on any size variable. However, the quantification of fishing activity was based on a ranked scale that determined only the type of fishing (commercial or recreational) and not its intensity. Accordingly, we cannot exclude the possibility that the four categories were too coarse to detect subtle impacts of fishing on size structure.

In addition to fishing pressure, the impact of shoreline modifications and the intensity of recreational activities had no influence on size structure. The effects of lake morphometry and nutrient status, both important in determining habitat complexity and habitat availability for fish, had a much stronger impact on the size structure compared with anthropogenic effects of shoreline development and recreational activities. We suggest that the categorical data on lake-use intensity are not sufficiently sensitive to detect the changes in size structure and that the degree of anthropogenic degradation of the German lakes is too low (Mehner *et al.*, 2005) to impact size structure significantly.

In summary, the application of size-related variables to multi-mesh gillnet catches is a promising tool that can provide important information on systematic shifts in the size structure of lake fish assemblages along environmental gradients. In particular, the measure of size diversity might be a powerful tool for analysing fish size distributions as it condenses

many different aspects of other size metrics into a single comparable value. The exponent  $c$  of the Pareto type II mass spectra uncovers differences in the relative abundances of small- and medium-sized fish.

However, we recommend the visualisation of size distributions (for instance in the form of size-frequency histograms) to complement the interpretation of results of the size metrics, because similar values can be obtained from different underlying size distributions. Our results suggest that the more frequent use of size-based approaches could help in evaluating the ecological status of lakes. Further studies including a broader range of environmental and geographical gradients would be beneficial for analysing the subtle response of size-related variables of fish assemblages.

### **1.5 Acknowledgments**

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## 2) European Study (8 countries)

### 2.1 Introduction

The management of freshwater ecosystems is increasingly based on the assessment of their ecological integrity (Scrimgeour & Dan Wicklum, 1996). Human activities have altered physical, chemical and biological processes in ecosystems which can be measured on the resident biological communities (Karr, 1991). However, biological indicators must be carefully selected in order to assess the impact of anthropogenic pressures. With the implementation of the Water Framework Directive (WFD 2000/60EC) in Europe, the development of sensitive biological metrics that indicate ecological status of surface waters, while simultaneously being comparable and applicable across large geographical scale, is important. The ecological status of rivers and lakes has to be assessed, *inter alia* via analyses of the composition, abundance and age structure of the fish fauna. However, harmonisation of taxonomic fish metrics might be challenging because species composition (alpha and beta diversity) can differ substantially between aquatic systems (Leprieur *et al.*, 2011), and native fish species richness is comparatively low in western and northern European lakes (Tammi *et al.*, 2003; Griffiths, 2006; Brucet *et al.*, submitted). Additionally, the development of sensitive taxonomic metrics can be hampered by the sampling systems. For example, the standardised sampling of fish in lakes by multi-mesh gillnets (CEN, 2005) was shown to be inadequate for rare and small species primarily living in shallow nearshore zones (Diekmann *et al.*, 2005).

In order to compare fish assemblage structures over large geographical scales (Hoeinghaus *et al.*, 2007), ecological guilds of fishes have been integrated into the functional description of lake fish assemblages in Denmark (Søndergaard *et al.*, 2005) and France (Launois *et al.*, 2011). However, high levels of ecological plasticity (Griffiths, 2006) and frequently observed ontogenetic niche shifts (Werner & Gilliam, 1984) could be an obstacle for a precise functional classification of European lake fish species. To avoid problems associated with the structural and functional complexity of fish assemblages, the use of body size, regardless of any taxonomic grouping, can be an alternative to develop sensitive metrics (Petchey & Belgrano, 2010). Additionally, information on the size structure of fish can be used as a proxy for the age structure (Gulland & Rosenberg, 1992), which explicitly has to be analysed for the assessment of freshwater fish assemblages according to the WFD. However, so far size of fish has primarily been considered only as an additional metric in systems developed to assess local, species-poor assemblages in coldwater streams (Breine *et al.*, 2004; Logez & Pont, 2011).

In particular, the size of fish may be a sensitive trait to indicate anthropogenic influences on lake ecosystems. For example, abundance of large-sized fish declines in response to oxygen depletion (Casselman & Harvey, 1975) or because of size-selective fishing (Gassner *et al.*, 2003). Furthermore, analyses within several European countries (Holmgren & Appelberg, 2000; Jeppesen *et al.*, 2000; Emmrich *et al.*, 2011) have shown that the size structure of lake fish populations and multi-species assemblages is sensitive to changes in water quality, showing an overall increase of small fish with increasing eutrophication. Besides anthropogenic disturbances such as eutrophication or fishing, a primary predictor of fish body size is temperature, which becomes important for large-scale studies. Temperature has a significant impact on organism size through physiological constraints at high temperatures also for ectothermic animals, including fish (Lindsey, 1966; Atkinson & Sibly, 1997). Fish assemblages in warm environments generally consist of more small-sized species (Knouft, 2004; Teixeira-de Mello *et al.*, 2009; Griffiths, 2011), and even individuals of the same species are larger in cold environments compared to their conspecifics in warm environments (Blanck & Lamouroux, 2007; Lappalainen *et al.*, 2008; Jeppesen *et al.*, 2010). However, a systematic comparison of the size structure of lake fish assemblages independently of species composition across a large geographical scale and an evaluation of their main environmental predictors remain to be made.

We analysed data from benthic multi-mesh gillnet catches in 728 lakes covering a latitudinal range of 28° and a longitudinal range of 35° across Europe. Variability in the size structure of the fish assemblages was analysed in relation to the geographical location of the lakes, climate variables, variables of lake morphology and anthropogenic lake pressures (particularly related to eutrophication) by using boosted regression tree analysis. We hypothesised that (1) across a large geographic scale differences in the size structure of lake fish assemblages are primarily driven by temperature. However, we hypothesised further that (2) anthropogenic pressures, in particular eutrophication, significantly modify the size structure of fish assemblages, thus making size metrics a suitable tool to evaluate the ecological integrity of lakes across large geographical scales.

## **2.2 Methods**

### **2.2.1 Fish data**

A database of multi-mesh gillnet catches was used to analyse the size structure of European lake fish assemblages. Natural lakes and reservoirs (both termed 'lakes' in the following) were sampled between 1993 and 2010 largely in accordance with the European standard EN 14757 for gillnet surveys in lakes (Appelberg *et al.*, 1995; CEN, 2005). Stratified random

sampling with benthic multi-mesh gillnets (type Nordic: length 30 m; height 1.5 m; 12 panels of 2.5 m each with mesh sizes between 5 and 55 mm knot to knot) was applied during late summer and early autumn with a pre-defined number of nets per lake set randomly in each depth stratum depending on lake area and depth. Slightly different sampling protocols were applied for 55 German lakes with the number of nets split between seasons (Mehner *et al.*, 2005). Some lakes were sampled with a reduced effort due to conservation and public acceptability issues (Winfield *et al.*, 2009). Pelagic fish catches were ignored because pelagic nets were not consistently used between the countries.

For the calculation of size metrics, information on individual fish size is essential to obtain a representative picture of the size structure of the fish assemblage. We selected only those lakes for which information on individual length (nearest cm) and/or fresh weight (FW; nearest g) was available for at least 95% of all fish.

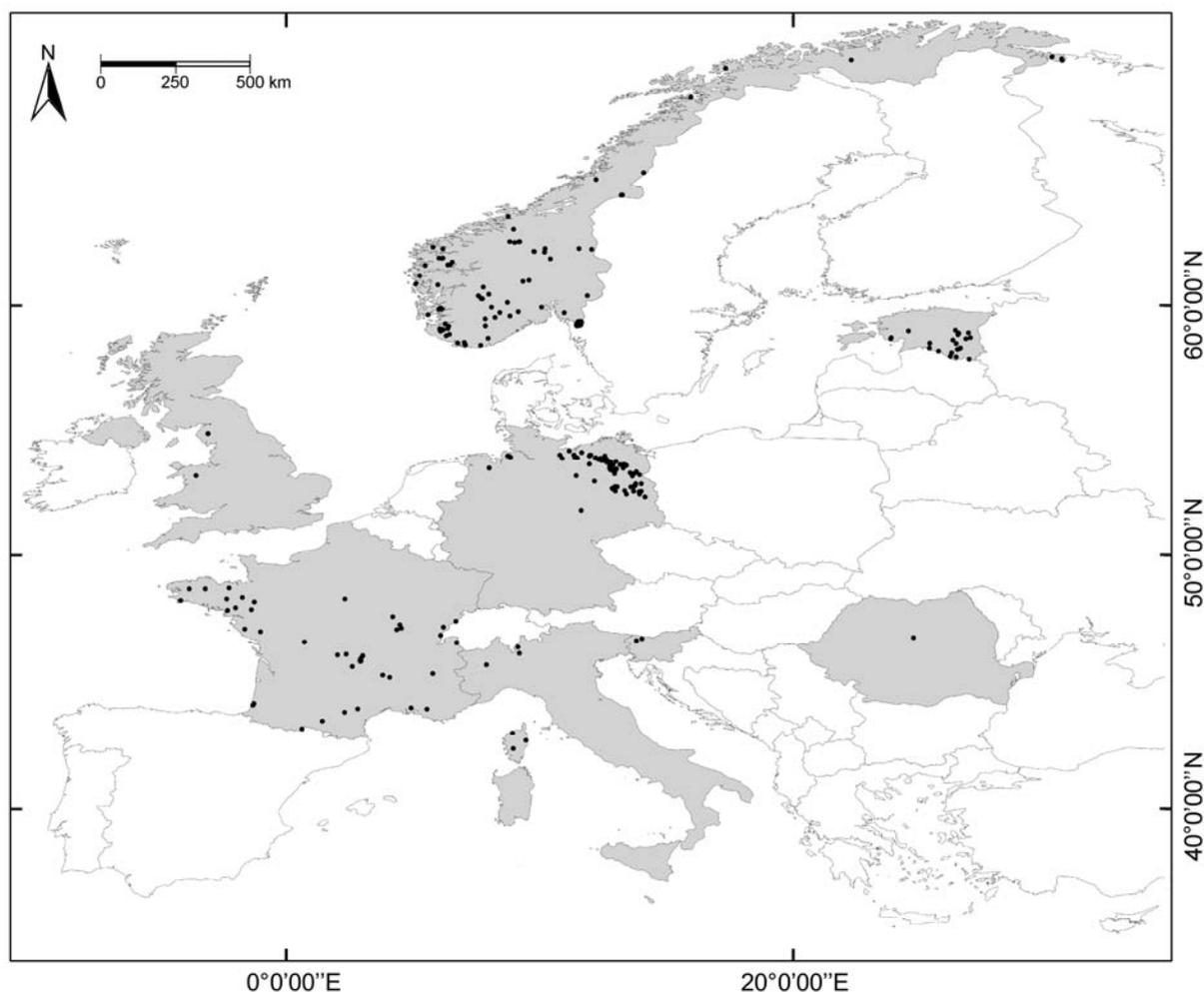


Figure 9: Geographical distribution of the 268 lakes across eight European countries (grey-coloured) with complete information on individual length and fresh weight of the benthic multi-mesh gillnet catches.

If only FW was recorded during field campaigns, length was estimated using latitudinal- and species-specific weight-length relationships (5.1% of the data) calculated from fish in the database with complete information on individual length and FW (Emmrich unpublished). The use of back-calculated length had no significant impact on the size distribution which was evaluated on a subset of 268 lakes with complete information on individual length and FW (Fig.9).

Size distributions of the original lengths were tested against the size distribution of back-calculated lengths from the original FW values using Wilcoxon test for paired data. In only one lake, the difference between the observed and back-calculated length distribution was significant ( $P = 0.05$ , Fig. 10). Due to biased sampling efficiency of multi-mesh gillnets for very small fish (Olin *et al.*, 2009; Prchalová *et al.*, 2009), all fish < 8 cm were removed in order to reduce the ‘noise’ of varying seasonal recruitment success and differences in sampling time and hence length of effective growing season. Furthermore, only those lakes were selected for which the catch size was  $\geq 50$  individuals to ensure that size metrics were not heavily influenced by outliers within small sample sizes.

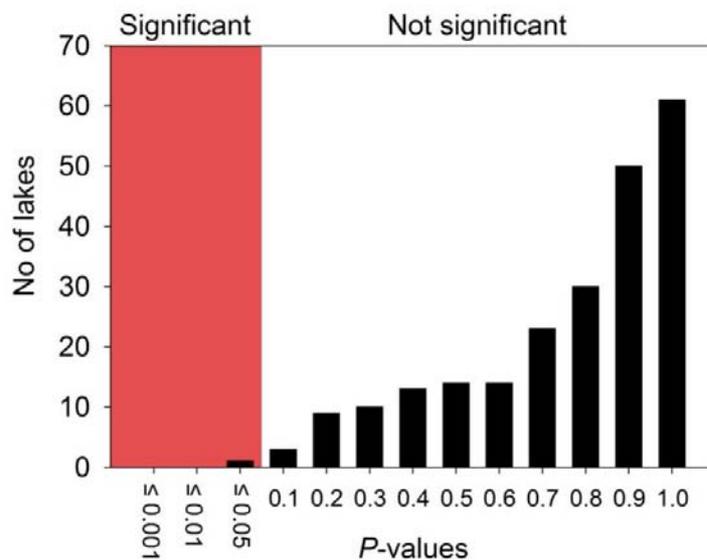


Figure 10: Distribution of  $P$ -values resulted from Wilcoxon tests for paired data to test whether fish length distributions back-calculated from original fresh weight values were significantly different (shaded part) from the original length distribution ( $n = 268$  lakes).

Finally, only lakes with  $\text{pH} \geq 6$  were included to avoid the interfering effects of acidification on the fish assemblage size structure. The final dataset comprised fish data from 728 lakes (642 natural lakes and 86 reservoirs) with perch and roach dominating the catches in most countries (Table 5; Fig. 11).

Table 5: Number of lakes sampled and number of species in the multi-mesh gillnet catches (after removing individuals < 8 cm).

Country	No. of lakes	No. of species	% roach	% perch
Sweden	444	28	31.4	56.8
France	127	40	34.8	19.8
Germany	75	30	32.9	40.0
Norway	49	17	11.8	59.3
Estonia	20	12	45.7	28.0
United Kingdom	8	11	25.7	60.1
Italy	3	12	7.2	58.9
Slovenia	2	16	31.6	50.5
Total	728	55	32.0	43.0

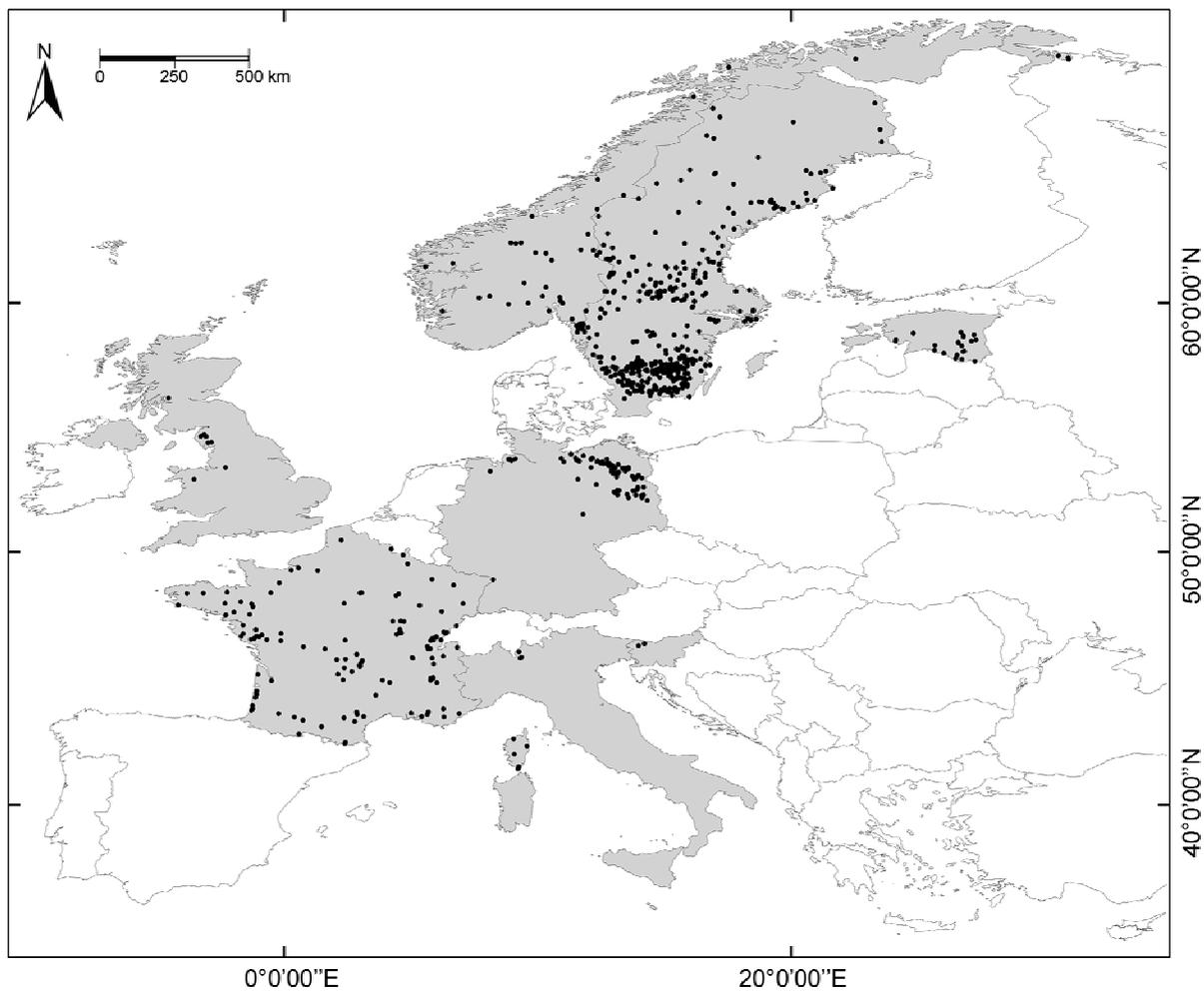


Figure 11: Geographical distribution of the 728 lakes across eight European countries (grey-coloured) whose fish assemblages were sampled with benthic multi-mesh gillnets.

## 2.2.2 Predictor variables

In total, 22 environmental predictor variables were used in this study (Table 6).

Table 6: Predictor variables used in boosted regression tree analyses. Type (cont = continuous; cat = categorical; bin = binary), range, mean and coefficient of variation (CV) are listed. Categorical pressures are ranked with the lowest categories representing lowest population densities resp. no shoreline bank modifications.

Variable (unit)	Type	Range	Mean	CV (%)
<b>Sampling effort</b>				
Number of benthic nets	cont	1 – 101	21.0	64.2
<b>Geographical location</b>				
Latitude	cont	41.40 – 69.70	56.1247	10.1
Longitude	cont	-4.62 – 30.78	12.5314	46.9
Altitude (m)	cont	-1 – 1739	233.1	104
<b>Climate</b>				
Average monthly temperature (°C)	cont	-3.7 – 15.7	5.9	55.4
Minimum mean monthly temperature (°C)	cont	-16.7 – 9.8	-3.4	137
Maximum mean monthly temperature (°C)	cont	6.8 – 23.1	15.7	14.4
Amplitude air temperature (°C)	cont	9.9 – 29.5	19.0	16.2
<b>Lake morphology</b>				
Area (km <sup>2</sup> )	cont	0.02 – 113	2.0	308
Mean depth (m)	cont	0.5 – 97.2	6.0	122
Maximum depth (m)	cont	1.0 – 190	16.7	107
<b>Nutrient status / lake pressures</b>				
pH	cont	6.0 – 10.0	7.1	11.6
Total phosphorus ( $\mu\text{g L}^{-1}$ )	cont	1.0 – 516	28.6	178
Agricultural land cover in catchment (%)	cont	0 – 97.9	24.9	117
Natural land cover in catchment (%)	cont	2.1 – 100	71.2	43.1
Population density in catchment	cat	1 – 4		
Shoreline bank modification	cat	1 – 5		
Upstream impoundment (yes, no)	bin			
Loss of downstream connectivity (yes, no)	bin			
Water-level fluctuation (yes, no)	bin			
Stocking (yes, no)	bin			
Type (natural, artificial)	bin			

Lake location was characterised using latitudinal and longitudinal coordinates and altitude (m a.s.l.). Monthly mean air temperature variables on the lake's location were obtained from a climate model with a spatial resolution of 10' latitude/longitude and taking into account elevation differences (New *et al.*, 2002). The temperature amplitude (difference between

mean temperature in July and January) was used as a proxy for seasonality. Lake morphology was characterised by area (km<sup>2</sup>), mean and maximum depth (m). Anthropogenic pressures included eutrophication variables (annual mean total phosphorus concentration in the lake ( $\mu\text{g L}^{-1}$ ), percentage of natural and agricultural grounds (CORINE land cover; CLC) and human population density in the catchment (inhabitants km<sup>-2</sup>; four classes). Shoreline-bank modifications were assessed by local experts on a ranked scale from 1 (no modifications) to 5 (highly modified). Hydrological modifications were evaluated on a binary scale (presence, absence) and included the existence of upstream impoundments, loss of downstream connectivity and occurrence of significant, anthropogenic-induced water level fluctuations. Finally, influence of stocking on the natural fish fauna based on expert opinion and lake type, whether natural or artificial (created by damming), were included in the analyses. Because of the large variability in size (0.02-113 km<sup>2</sup>) of the lakes sampled, the sampling effort varied strongly as well (1-101 benthic nets per lake). Therefore, we additionally evaluated the effect of sampling effort on the size metrics and added the number of benthic nets set in each lake to the predictor variables.

### 2.2.3 Size metrics

Choice of size metrics was based on a study in 78 German lakes (Emmrich *et al.*, 2011, see part 1 of this report). For all European lakes included, we calculated geometric mean length (cm), variance of the length data, skewness and kurtosis of the length-frequency distribution, number of size classes, maximum length (cm), interquartile range (cm), length at the 95<sup>th</sup> percentile (cm) and size diversity. Size diversity (Quintana *et al.*, 2008) combines several aspects of other size metrics into a single comparable value and was tested to be sensitive to a broad range of lake descriptors at the regional scale (Emmrich *et al.*, 2011). For fish-length data, size diversity is high when the catch consists of many different size classes (single size classes can still dominate the catch) or if the abundances between the size classes are relatively equal (Emmrich *et al.*, 2011). For details of all other size metrics see Emmrich *et al.* (2011). Size metrics related to fish weight could not be calculated for the European dataset, because of missing FW data in 61% of the lakes. Additionally, we did not include normalised length spectra, as for almost one third of the lakes the  $R^2$  of the linear regression models was  $< 0.5$ .

### 2.2.4 Data analyses

We used boosted regression tree (BRT) analysis, a predictive technique combining boosting algorithms with regression trees to model nonlinear relationships and interactions (Friedman,

2002; De'ath, 2007). BRTs were applied to estimate the contribution of each predictor to the total variation in each of the size metrics. Interactions between predictors are automatically included in the models via tree complexity. Similar to other tree-based methods, BRTs can simultaneously handle categorical and continuous data and allow the incorporation of missing data. Predictor variables do not need to be transformed, outliers need not be eliminated and predictors can strongly correlate (Breiman *et al.*, 1984; De'ath, 2007). A Gaussian error distribution was selected for all BRT models. Predictive performance of the BRT models was evaluated using ten-fold cross-validation. Ten mutually exclusive subsets were randomly selected and model predictions were compared to withhold proportions of the data. The optimal number of trees (nt) producing the lowest prediction error without model overfitting was identified by combining several levels of tree complexity (tc) (tested values of the parameters in brackets) (1-6), learning rate (lr) (0.05, 0.01, 0.005, 0.001) and bag-fraction (bf) (0.5, 0.75). To reduce the amount of contribution from individual trees to the final model, the minimum limit to fit the models was set to 1000 trees (Elith *et al.*, 2008). Redundant predictor variables may increase variance and thus reduce model accuracy (De'ath, 2007). Consequently, models were simplified by removing non-informative predictors following Elith *et al.* (2008). Final parsimonious models were computed with the reduced set of predictor variables and the optimal combinations of nt, tc, lr, and bf. BRT analysis does not generate *P*-values, but the relative influence (a measure of how often a predictor variable is selected and what is its influence on model improvement) is used to estimate the significance of each predictor. We considered predictors with a relative influence of  $\geq 10\%$  to be important in this study. Pair-wise interactions between predictor variables were quantified according to Elith *et al.* (2008). Finally, partial dependence plots were used to visualize the marginal effects of individual predictor variables on the response after accounting for the average effects of all other predictor variables (Friedman, 2002).

Complete information on eutrophication (total phosphorus, CLC agricultural) was only available for 53.2% of the lakes, but this is not a problem in BRT analysis due to the use of dummy values. However, to evaluate whether the dummy values changed the relative influence of predictor variables, we re-ran the models with a reduced set of lakes ( $n=378$ ), comparable in geographical range (Fig. 12), for which eutrophication variables were available.

Patterns of the size metrics identified in the BRT analyses were subsequently analysed using ANOVA and post hoc t-tests in cases of significant ANOVA results. Analyses were performed in R (R Development Core Team, 2008) version 2.8.0 using the gbm package (Ridgeway, 2006) and the code of Elith *et al.* (2008).

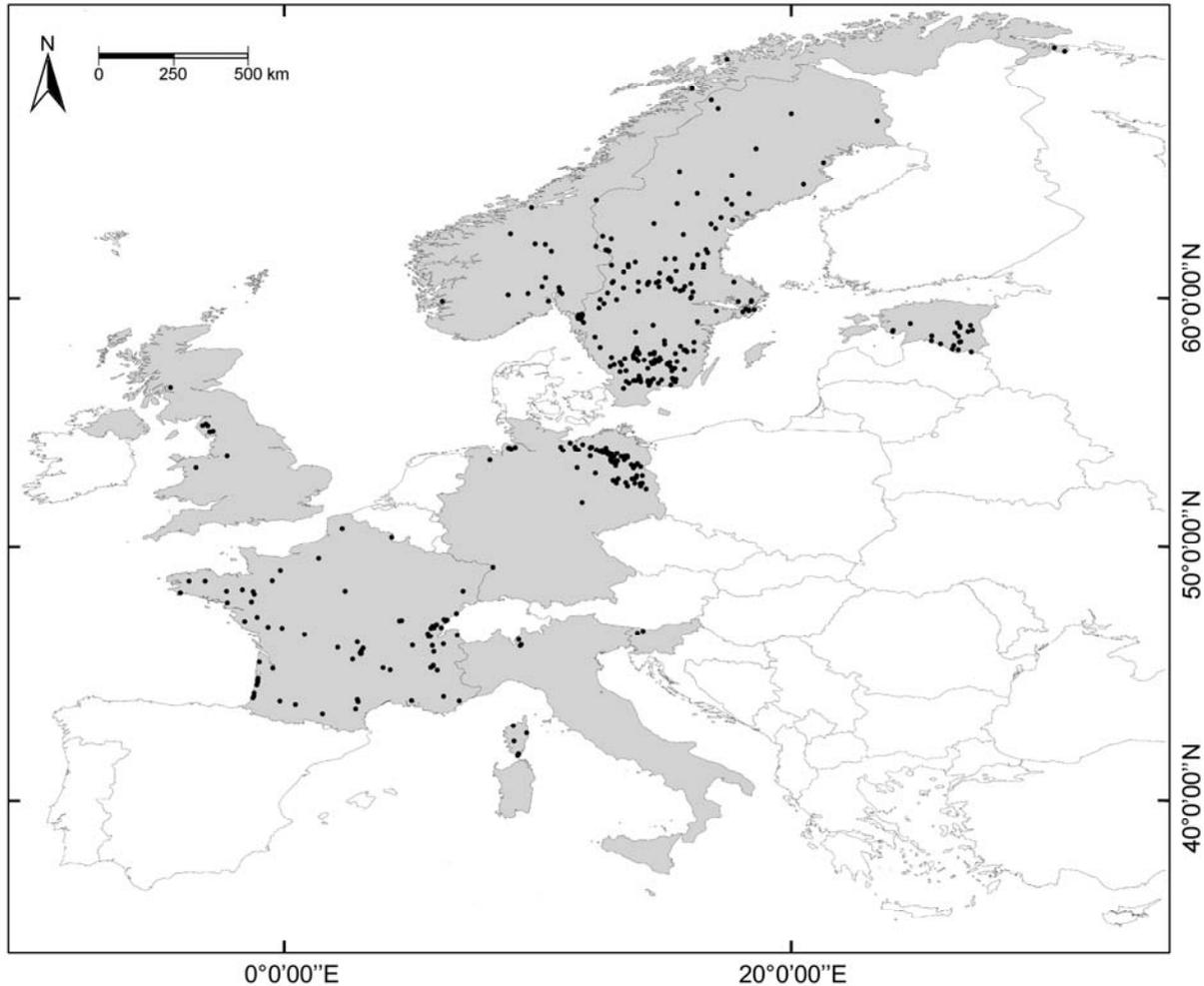


Figure 12: Geographical distribution of the 378 lakes across eight European countries with information on total phosphorus concentration.

## 2.3 Results

### 2.3.1 Size metrics

Many size metrics were strongly correlated and highly variable (Table 7) and BRT analyses indicated high sensitivity of skewness, kurtosis, number of size classes and interquartile range towards sampling effort (relative influence of number of nets > 10%; Table 8; Fig.13). Consequently, we selected only three weakly correlated (Spearman's  $r < 0.34$ ) metrics for further analyses that were obviously less sensitive to sampling effort, namely geometric mean length (GM), maximum length ( $L_{max}$ ) and size diversity (SD). Variability in these three metrics was substantial between the 728 lakes. Average geometric mean length of all fish assemblages was 14.1 cm ( $\pm 2.6$ ; 9.6-32.0 cm) (standard deviation; range). Maximum length averaged 54.4 cm ( $\pm 15.9$ ; 19-120 cm) and average size diversity was 1.8 ( $\pm 0.3$ ; 0.4-2.7) (Table 7).

Table 7: Correlation matrix of the nine size metrics. The white matrix contains Spearman's  $r$  values; the grey matrix contains the corresponding  $P$ -values. Furthermore minimum, mean and maximum values of the size metrics are given.

	GM	VAR	SKEW	KURT	NSC	IQR	$L_{\max}$	$L_{95}$	SD
GM	---	0.308	-0.392	-0.451	0.024	0.465	-0.043	0.566	0.169
VAR	<0.001	---	-0.475	-0.311	0.537	0.576	0.525	0.786	0.785
SKEW	<0.001	<0.001	---	0.936	-0.704	-0.678	-0.335	-0.590	-0.664
KURT	<0.001	<0.001	<0.001	---	-0.508	-0.673	-0.252	-0.434	-0.558
NSC	0.514	<0.001	<0.001	<0.001	---	0.324	0.549	0.473	0.534
IQR	<0.001	<0.001	<0.001	<0.001	<0.001	---	0.165	0.606	0.778
$L_{\max}$	0.249	<0.001	<0.001	<0.001	<0.001	<0.001	---	0.193	0.341
$L_{95}$	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	---	0.577
SD	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	---
Minimum	9.6	3.7	-0.3	-1.8	8.0	1.0	19.0	12.0	0.4
Mean	13.6	30.9	1.0	-0.3	27.5	6.0	53.0	23.0	1.8
Maximum	32.0	198.0	3.8	15.1	54.0	22.0	120.0	50.0	2.7

GM = geometric mean length; VAR = variance; SKEW = skewness of the length-frequency distribution (LFD), KURT = kurtosis of the LFD; NSC = number of size classes; IQR = interquartile range;  $L_{\max}$  = maximum length;  $L_{95}$  = length at the 95<sup>th</sup> percentile of the LFD; SD = size diversity.

Table 8: Contribution of predictors (relative influence in %) of the boosted regression tree analyses to the nine size metrics ranked in order of decreasing median contribution. For full names of the size metrics see Table 7.  $T_{\max}$  = maximum temperature;  $T_{\text{amp}}$  = amplitude temperature;  $T_{\text{ave}}$  = average temperature; CLC = Corine Land Cover.

Predictor	GM	VAR	SKEW	KURT	NSC	IQR	$L_{\max}$	$L_{95}$	SD	Median
Latitude	15.51	13.74	7.86	5.65	12.98	7.24	13.06	12.26	12.05	12.26
Longitude	4.82	16.62	6.88	10.69	7.49	9.15	10.68	11.93	15.83	10.69
Area	3.06	5.20	19.09	18.73	20.98	18.07	7.39	4.80	9.80	9.80
Altitude	10.86	16.45	5.90	7.30	2.73	5.72	14.48	16.91	7.19	7.30
Number nets	0.51	3.95	28.97	21.47	32.06	17.72	4.65	3.06	6.50	6.50
pH	5.63	4.87	6.32	7.50	3.82	5.32	8.87	6.10	5.90	5.90
Maximum depth	12.26	4.84	4.99	5.97	3.33	5.08	7.68	11.28	5.66	5.66
Mean depth	3.41	6.79	4.86	4.47	3.03	4.61	8.54	5.93	10.50	4.86
$T_{\max}$	28.30	4.65	2.25	2.96	1.34	4.03	7.30	9.49	3.98	4.02
$T_{\text{amp}}$	3.03	4.22	2.15	3.42	1.38	3.09	4.12	3.56	4.08	3.42
CLC natural	2.61	4.04	3.34	2.44	1.87	4.84	2.69	3.57	3.30	3.30
TP	1.67	1.36	2.57	3.13	1.83	5.69	3.29	3.17	4.12	3.13
$T_{\min}$	3.12	8.26	1.21	3.00	2.68	3.94	2.95	2.71	4.57	3.00
CLC agriculture	2.50	2.12	2.06	0.86	3.21	2.67	2.03	2.42	4.20	2.42
$T_{\text{ave}}$	2.71	2.89	1.55	2.41	1.27	2.83	2.27	2.81	2.32	2.41

### 2.3.2 Boosted regression tree models

Final BRT models were run with learning rates of 0.005 and 0.001, tree complexities of one ( $L_{max}$ ), three (GM) and four (SD) and a bag fraction of 0.5. Optimal number of trees ranged between 1200 and 3750. Analyses on model simplification indicated that categorical and binary predictors of anthropogenic pressures had only negligible effects on the predictive performance of the BRT models. Consequently, we removed all categorical and binary predictors from the final models. Predictive performance of the simplified models including the 15 continuous predictors was 23.5% ( $L_{max}$ ), 25.3% (SD) and 36.0% (GM). Cross-validation correlation coefficients  $> 0.4$  indicated reliable output of the BRT models. The BRT models based on the reduced set of lakes (complete eutrophication variables available) resulted in comparable model predictions, except for  $L_{max}$  for which a reduction of the predictive performance from 23.5% to 11.8% was observed (predictive performance GM: 36.2%; SD 28.5%).

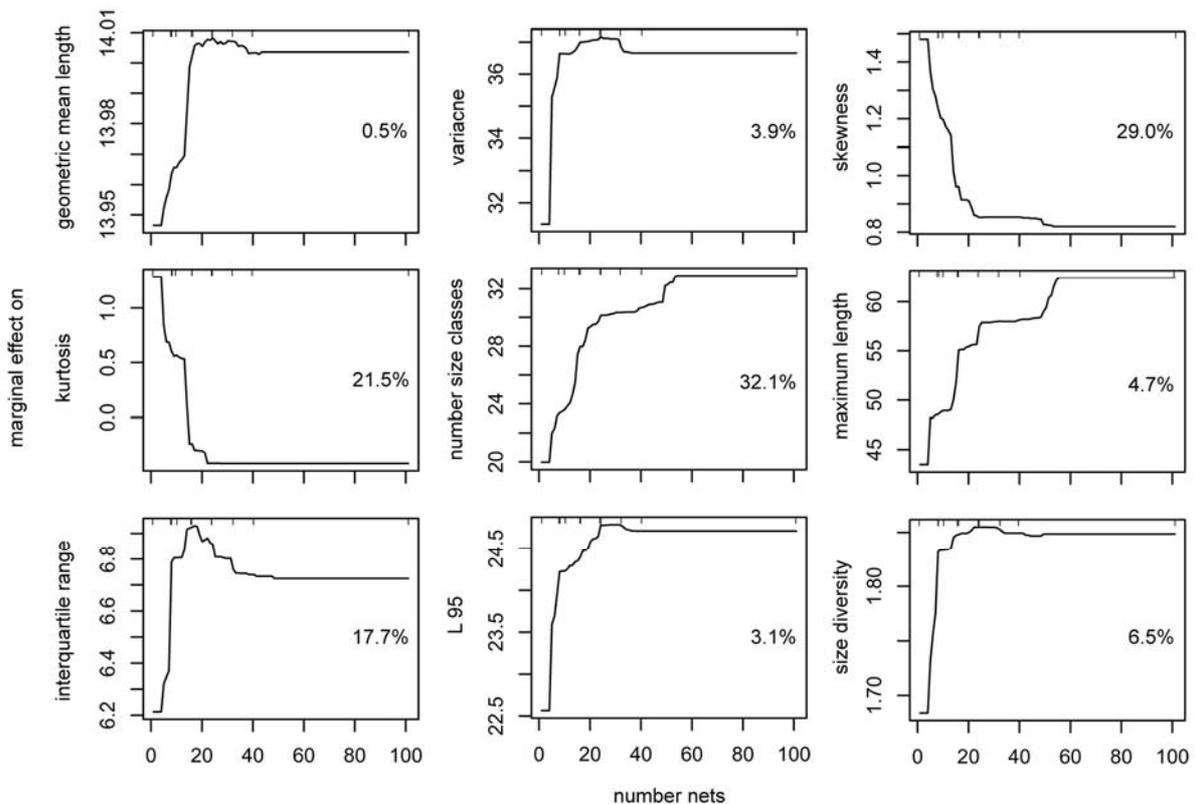


Figure 13: Partial dependence plots showing the marginal effect of sampling effort (number of benthic multi-mesh gillnets) on the size metrics. Rug plots on the top horizontal axes indicate the distribution of sampling effort, in deciles. Apparently, only 8 ticks are visible, because three deciles included only one net number ( $n = 8, 16, 24$ ). Percentage values indicate the relative importance of sampling effort to each size metric in the boosted regression tree models.

### 2.3.3 Influence of predictors and interactions between predictors

Contribution of single predictors to the BRT models was similar among the three size metrics (see small standard errors Fig. 14). Geographic variables (latitude, longitude, altitude) and maximum temperature showed the strongest correlations to variation in the size metrics. Differential effects on the three size metrics were observed only for maximum temperature (relative influence on GM 28.3%, on  $L_{max}$  7.3%, on SD 4.0%). Furthermore, lake depth (maximum and mean depth) was important (Fig. 14a, b). Sampling effort had only a weak influence on GM (0.5%), but a slightly stronger influence on  $L_{max}$  (4.7%) and SD (6.5%) (Table 8; Fig.13). All other predictors had only a weak mean relative influence ( $\leq 7\%$ ). Notably, the relative influence of all anthropogenic pressures indicating eutrophication such as total phosphorus concentration and the percentage of agricultural ground in the catchment was very low ( $< 4.3\%$ ) (Fig.14a). The importance and rank of predictors did not change substantially when tested on the reduced dataset (Fig. 14b), suggesting that the missing data on eutrophication had no effect on the dominant predictors of fish-size structure.

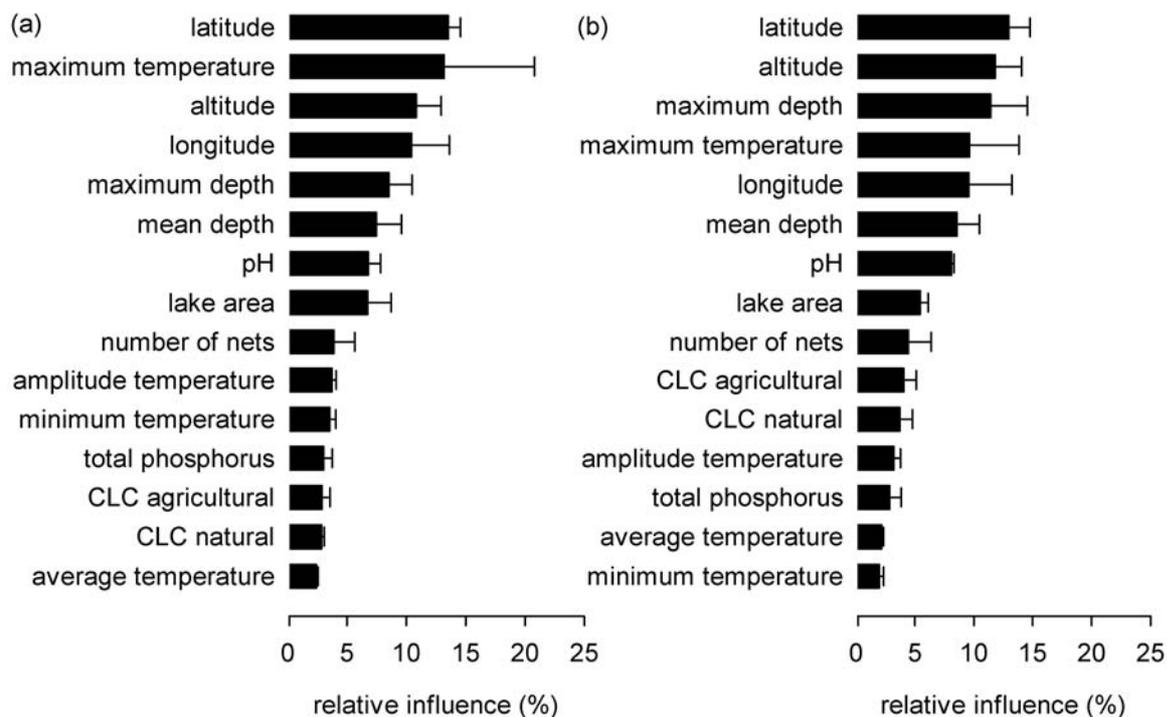


Figure 14: Mean relative importance (+ standard error) of continuous predictor variables to the three size metrics in the boosted regression tree analysis. Predictor variables are ranked in order of decreasing mean values. CLC = CORINE land cover, a: full dataset of 728 lakes; b: reduced dataset of 387 lakes with complete information on eutrophication pressures.

The functions fitted to the BRT models were highly variable in shape and showed both linear and non-linear patterns (Fig. 15). GM decreased with increasing maximum temperature and increased with latitude, altitude and maximum lake depth. Interactions were straightforward, showing the relationship of decreasing maximum temperature at increasing latitude and

increasing GM (Fig. 16).  $L_{max}$  and SD were lowest at mid latitudes (53-62°) and increased further north and south. Additionally, both metrics decreased with increasing longitude (Fig. 315).  $L_{max}$  was further influenced by altitude with the highest values occurring between 500 and 1000 m (Fig. 15).

Mean depth of lakes had a significant impact only on SD. The lowest values were found in lakes of approximately 2.5 m depth. Slightly higher values were observed in ultra-shallow lakes, and the highest SD was found in lakes > 8 m depth (Fig. 15). Interactions showed that SD was highest in the northern and southern regions of Europe characterised by a relatively constant seasonal climate (low amplitude in temperature, Fig. 16).

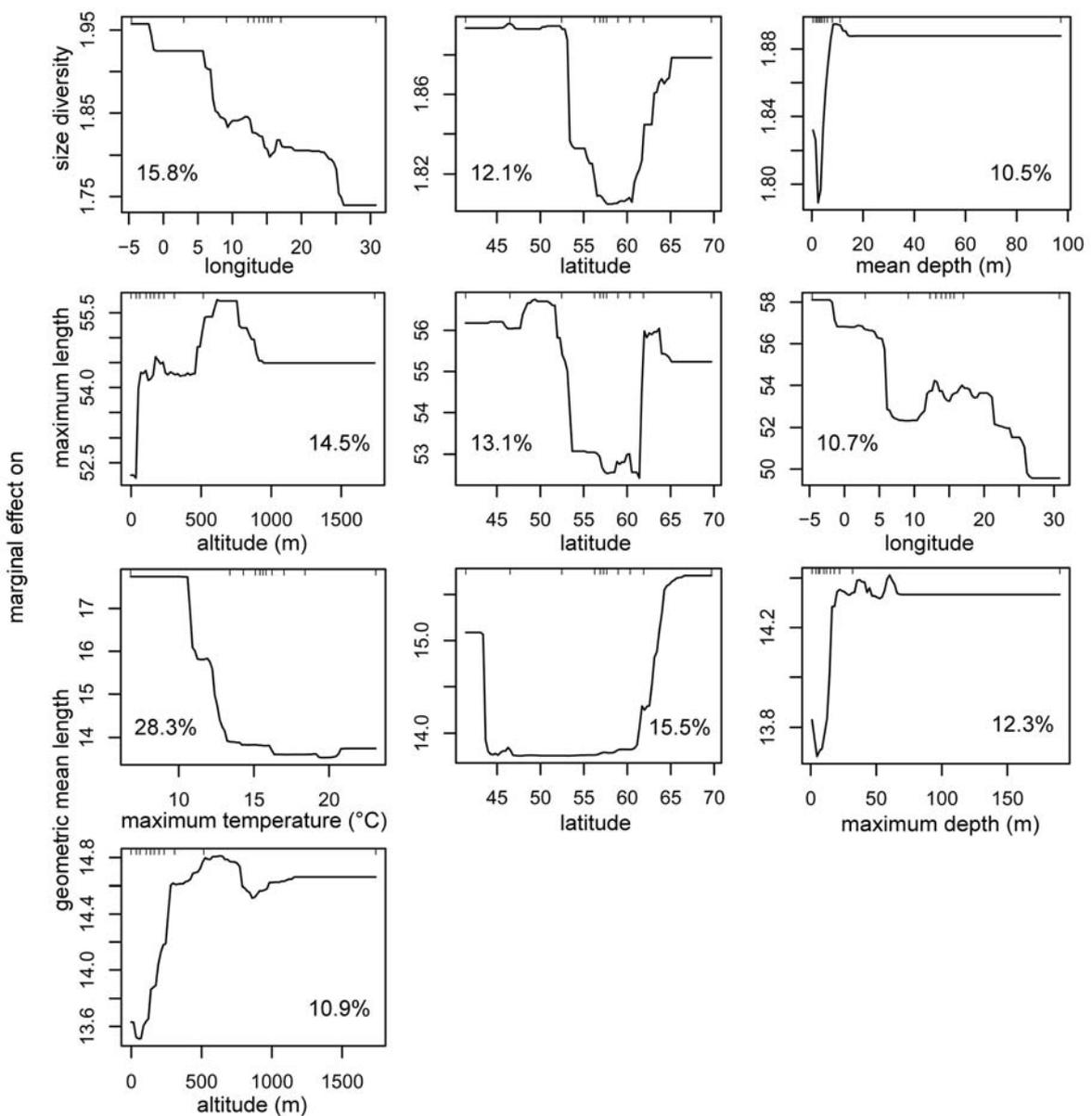


Figure 15: Partial dependence plots for the most influential predictor variables on size diversity, maximum length and geometric mean length. Rug plots on the top horizontal axes indicate the distributions of the predictor variables, in deciles. Percentage values indicate the relative importance of single predictor variables in the boosted regression tree models.

However, longitudinal patterns of  $L_{\max}$  and SD (Fig. 15) were biased through a significantly higher sampling effort (number of nets) in western Europe ( $< 7^\circ$  longitude) compared to middle ( $7-25^\circ$ ) and eastern Europe ( $> 25^\circ$ ) (ANOVA:  $F_{1,726} = 25.9$ ;  $P < 0.001$ ; post hoc t-tests:  $P < 0.01$ ) (Fig. 17a).

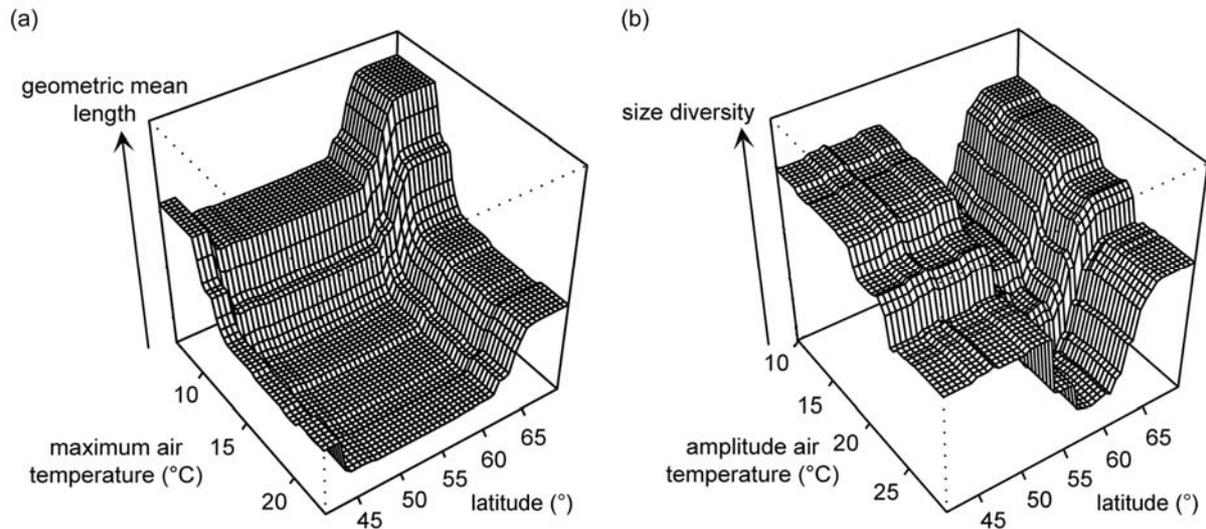


Figure 16: Three-dimensional mesh plots showing the strongest pair-wise interaction in the boosted regression tree model for geometric mean length (a) and size diversity (b). Arrows on the z-axes indicate direction of increasing values of the size metrics.

A higher net number significantly affected the number of different fish size classes (1 cm interval) caught in the multi-mesh gillnets (ANOVA:  $F_{1,726} = 225.6$ ;  $P < 0.001$ ; post hoc t-tests:  $P < 0.001$ ) (Fig. 17a, b). Latitudinal patterns were also influenced by differences in sampling effort with significantly more nets used in the large southern European lakes compared to the often smaller middle and northern European lakes included in the database (ANOVA:  $F_{1,726} = 27.9$ ;  $P < 0.001$ ; post hoc t-tests:  $P < 0.001$ ). This difference in effort resulted in more size classes of fish caught and, consequently, higher values of SD in southern European lakes (ANOVA:  $F_{1,726} = 130.6$ ;  $P < 0.001$ ; post hoc t-tests:  $P < 0.001$ ). Variations between middle and northern Europe were not significantly different (post hoc t-tests:  $P > 0.05$ ) (Fig. 17c, d), thus reflecting unbiased differences in the size structure of those fish assemblages.

Despite differences in sampling effort, the length-frequency distributions of fish from lakes pooled for each latitudinal class ( $< 53^\circ$ ,  $53-62^\circ$ ,  $> 62^\circ$ ) showed a consistent trend with a decreasing proportion of small fish further north, indicated by a decrease in skewness (southern Europe: 3.5, middle Europe: 2.7, northern Europe: 2.1) and kurtosis (southern Europe: 12.2, middle Europe: 6.4, northern Europe 2.9) (Fig. 18).

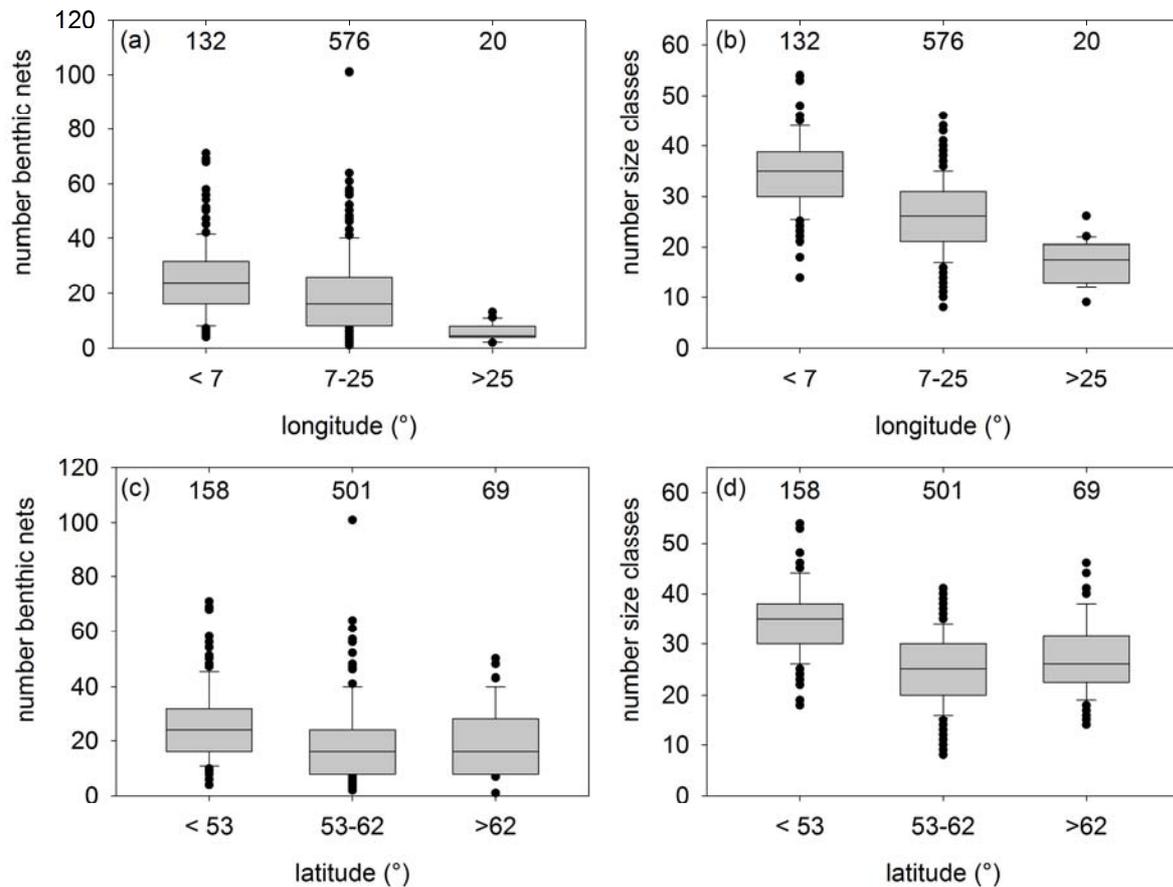


Figure 17: Box and Whisker plots showing the differences in sampling effort (number of benthic nets) and the corresponding differences in the number of size classes (1cm class interval) which impacted the results of the size metrics maximum length and size diversity. Classification of longitude and latitude based on stepwise changes in metric response in the marginal plots from the boosted regression tree analyses (Fig. 15). Plots show medians, 25<sup>th</sup> and 75<sup>th</sup> percentiles (boxes), 10<sup>th</sup> and 90<sup>th</sup> percentiles (whiskers) and outliers (dots). Number of lakes in each class is given on the top horizontal axes.

## 2.4 Discussion

The results from BRT analysis on three size metrics of 728 European lake fish assemblages support our hypothesis that large geographical scale variables related to temperature had the strongest influence on the size structure. However, contrary to our initial assumptions, eutrophication had, together with all other anthropogenic pressure variables tested, no significant impact on the size structure.

Furthermore, we showed that GM of the fish assemblage was the only size metric tested that could be directly compared among lakes without taking into account differences in sampling effort (*i.e.* number of nets). However, if lakes, strongly differing in area and depth, are sampled with benthic multi-mesh gillnets according to the European standard (CEN, 2005), differences in sampling effort can be substantial. The patterns of size metrics corresponding

to large fish in the catch (SD,  $L_{max}$ ) were thus partly drawn by differences in sampling effort, which makes a comparative analysis of the purely abiotic effects on these metrics difficult.

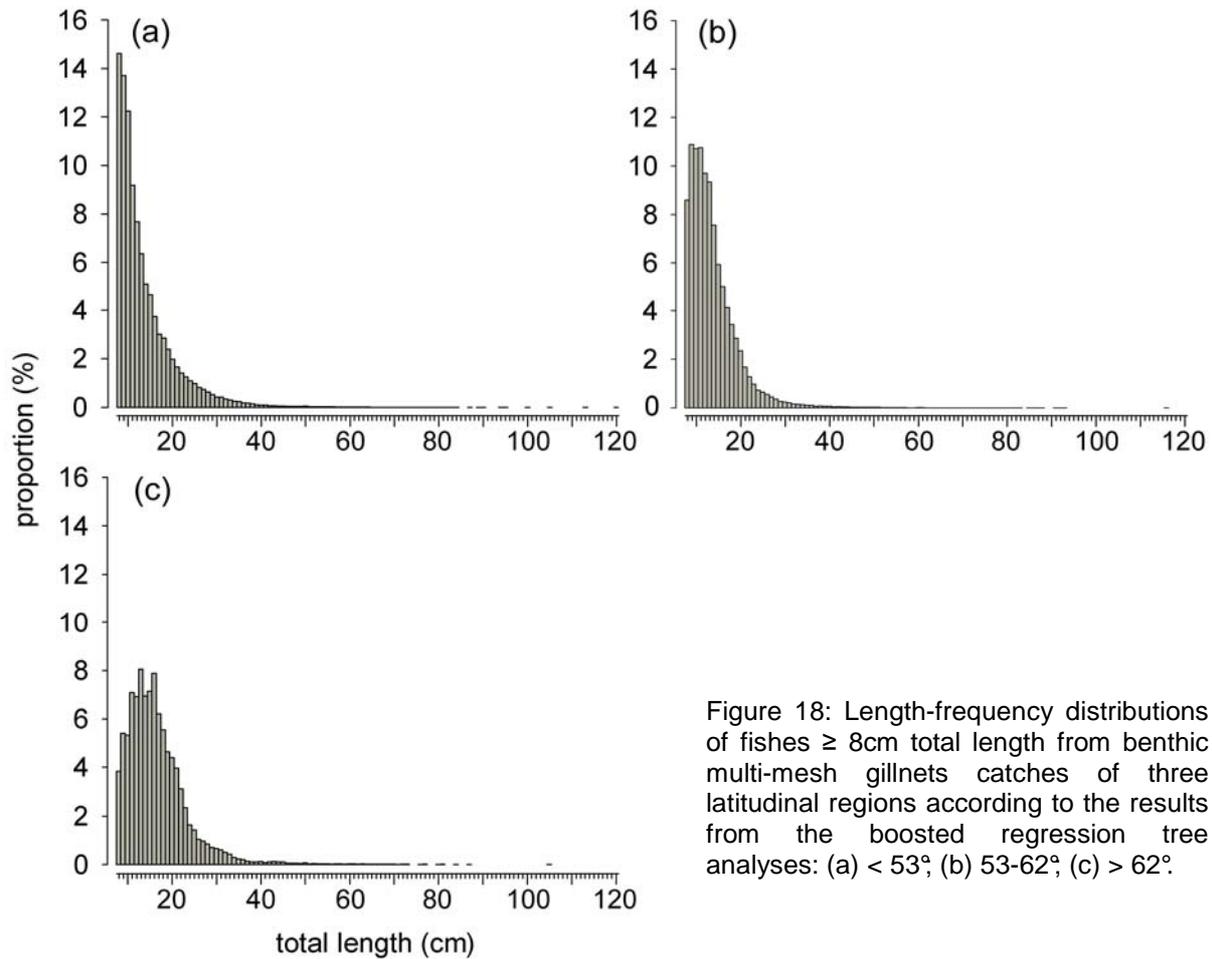


Figure 18: Length-frequency distributions of fishes  $\geq 8$ cm total length from benthic multi-mesh gillnets catches of three latitudinal regions according to the results from the boosted regression tree analyses: (a)  $< 53^\circ$ ; (b)  $53-62^\circ$ ; (c)  $> 62^\circ$ .

Temperature has a significant effect on the size of aquatic ectotherms from individual to assemblage level (Daufresne *et al.*, 2009). There is a strong biogeographic perspective on the body size of fish, primarily with a taxonomic focus, however. These approaches take into account differences in species-body size distributions (*i.e.* changes in species richness across size classes; Knouft, 2004; Griffiths, 2011) or intraspecific variations in fish body size across latitudinal gradients (Heibo *et al.*, 2005; Blanck & Lamouroux, 2007; Lappalainen *et al.*, 2008). By contrast, comparisons of geographical patterns on the size structure of fish assemblages are still rare (Jeppesen *et al.*, 2010; Emmrich *et al.*, 2011).

Our results showed that the size structure of lake fish assemblages, independently of any taxonomic grouping, is primarily influenced by temperature (measured as latitude, altitude, maximum mean air temperature) and lake depth. Lake depth and lake temperature are closely linked. Shallow lakes generally tend to be warmer compared to deep lakes (Choi, 1998). Consequently, all significant environmental predictors identified by the BRT analyses

can be directly linked to the environmental temperature of the fish assemblages. In cold environments, assemblages consisted of, on average, larger individuals. Furthermore,  $L_{\max}$  and SD were higher. Although we showed that patterns in  $L_{\max}$  and SD were partly influenced by differences in sampling effort, differences in the size metrics between middle and northern Europe were not seriously biased because of comparable numbers of nets used in these lakes.

$L_{\max}$  increased with altitude and was highest in southern and northern Europe. The high  $L_{\max}$  in southern lakes was the result of a significantly higher sampling effort in those lakes. However, patterns between middle and northern European lakes can be interpreted as the result of higher longevity and hence larger  $L_{\max}$  with increasing latitude, as observed for several European fish species (Blanck & Lamouroux, 2007). Substantial altitudinal effects were only observed between lowland lakes (< 90 m a.s.l.) compared to all lakes at higher altitudes. The lowland lakes were, on average, shallower, suggesting a higher thermal regime which, in turn, favours small-sized fish (Choi, 1998).

At high latitudes SD was high because of more uniform (bell-shaped) fish-length distributions in the gillnet catches (Emmrich *et al.*, 2011). Such length-frequency distributions are characteristic for, for instance, Arctic fish populations with stable size structures close to steady state that do not show strong fluctuations of single size classes over time (Johnson, 1994). The warmer (southern) lakes were generally sampled with more nets which, in turn, would result in high SD values due to a higher number of different size classes. However, the size structure of those fish assemblages was characterised by a negative exponential curve and in general small GM, indicating dominance of the small size classes even after excluding the fish < 8 cm from the catches. Such patterns are largely determined by a significant increase in the abundance of small-sized species and juvenile fish (Daufresne *et al.*, 2009; Teixeira-de Mello *et al.*, 2009).

In lakes, temperature and trophic status are closely linked via lake depth. Shallow (warm) lakes are in general nutrient rich (Nõges, 2009) and fish densities increase in number and biomass with increasing lake productivity (Jeppesen *et al.*, 1997; Brucet *et al.*, submitted). However, at comparable or even reduced nutrient concentrations, warm lakes still exhibit higher fish densities in particular of small-sized individuals compared to cold lakes (Downing & Plante, 1993; Jeppesen *et al.*, 2010; Brucet *et al.*, submitted). Temperature is the primary trigger of growth, maturation and life span. Fish in warm lakes characterised by less stable thermal regimes grow faster early in ontogeny, but slower later in ontogeny, mature earlier and have shorter life spans compared to fish in cold lakes with relatively stable thermal regimes (Choi, 1998; Blanck & Lamouroux, 2007; Jeppesen *et al.*, 2010). This explains the key influence of temperature on the size structure of fish assemblages when compared

across large geographic and climatic gradients. It might further explain why trophic status was such a poor predictor in our study. Variation in the trophic status of the lakes was most likely overridden by temperature effects. Comparisons of fish assemblages in Denmark and northern Germany, areas with less pronounced climatic variability, have identified a stronger influence of differences in lake productivity on the size structure of fish assemblages (Jeppesen *et al.*, 2000; Emmrich *et al.*, 2011). Except in some cases (Drake & Pereira, 2002; Launois *et al.*, 2011), response of fish metrics to hydromorphological alteration has seldom been shown (Mehner *et al.*, 2005; Bruce *et al.*, submitted). This can be the consequence of an overall weak impact of hydromorphological pressures on lake fish assemblages compared to other pressures or because of insufficient data quality (categorical data) of the pressure variables (Mehner *et al.*, 2005).

In this study, shoreline bank modifications showed no significant effect on the size structure of fish assemblages whatever the metric considered. This is in accordance with results from German lakes (Emmrich *et al.*, 2011) and indicates that sampling of lake-fish assemblages by benthic multi-mesh gillnets does not provide ideal data to evaluate the ecological integrity of lakes, at least across large geographic scales. Only non-taxonomic metrics related to the overall fish densities in lakes have proven to be sensitive to eutrophication pressures at a large scale (Bruce *et al.*, submitted). In contrast, taxonomic and size-based metrics were primarily influenced by lake morphology and climatic variability on a transnational scale (Mehner *et al.* 2007; Bruce *et al.*, submitted) and do therefore not reliably reflect the impact of anthropogenic pressures on the lakes at a large scale. Even dividing the lake set into smaller geographical regions according to the definition of the WFD geographical intercalibration groups (GIGs; similar to ecoregional division of Europe, Illies, 1978) for lakes (EC, 2005), BRT analysis gave comparable results with still weak relative importance (< 7%) of all pressure variables tested for the Northern GIG (n = 501 lakes) and the Central-Baltic GIG (n = 179 lakes) (Figure 19).

We also showed that differences in the size structure of benthic lake fish assemblages sampled according to the European standard (CEN, 2005) could not be considered independently of sampling effort. Although multi-mesh gillnets are designed to reduce size selectivity, the number of nets set in each lake significantly influenced all size metrics except GM if the range of nets used varied substantially. This influence becomes less significant if lakes are sampled with comparable effort, in particular if the minimum number of nets is not too low (Emmrich *et al.*, 2011). Furthermore, we emphasize that catches from pelagic nets were not included here (they were not consistently used among the countries), and pelagic catches likely have different size distributions (Jeppesen *et al.*, 2006; Lauridsen *et al.*, 2008) and might respond differently to changes in pressures.

Only the GM of the fish assemblages can be compared among lakes independently of the number of nets used because it is less influenced by positive outliers (large-sized fish).

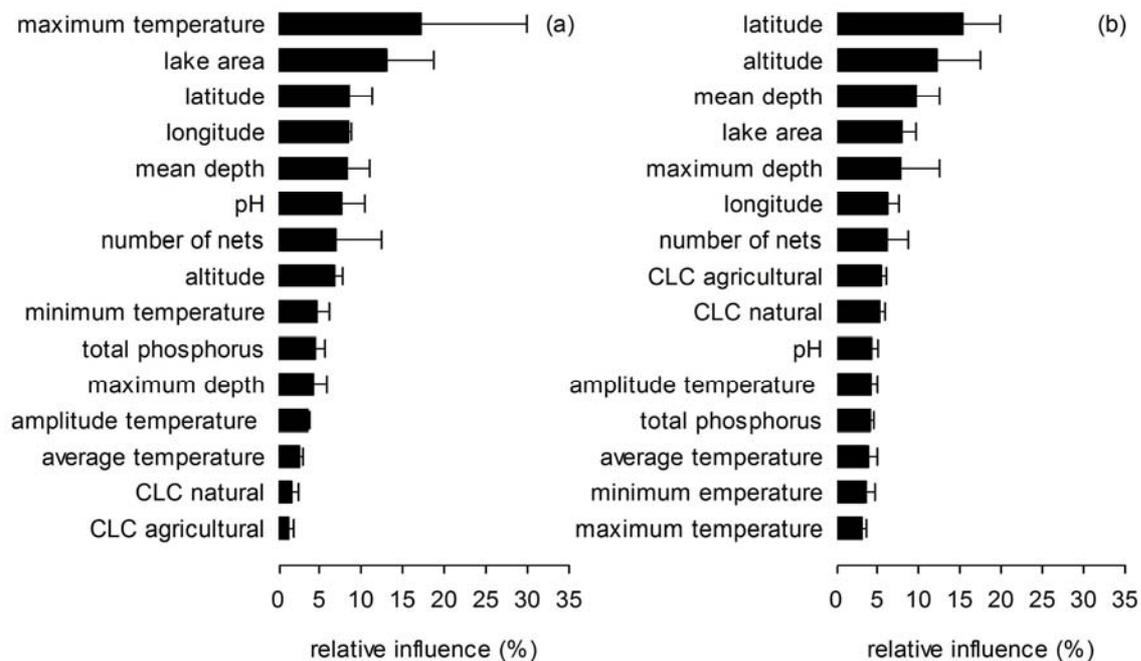


Figure 19: Mean relative importance (+ standard error) of continuous predictor variables to the three size metrics in the boosted regression tree analysis. Predictor variables are ranked in order of decreasing mean values. CLC = CORINE land cover, a: Nordic GIG (n = 501 lakes); b: Central-Baltic GIG (n = 179 lakes).

Size metrics providing information on the abundance of large-sized fish in a sample such as SD or  $L_{max}$  are biased if the sampling effort varies considerably. However, large fish are often of primary importance for the identification of large-scale disturbances such as impacts of fishing, non-native species invasion or climate change (Blanchet *et al.*, 2010; Greenstreet *et al.*, 2011). Our BRT analyses indicated that at least ten benthic multi-mesh gillnets (for some size metrics even more) are necessary, independently of lake area and lake depth, to obtain comparable information on the size structure of lake fish assemblages.

Nevertheless, although metrics characterising the size structure of lake fish assemblages will probably play no significant part in large-scale comparisons of ecological integrity according to the aims of the WFD, our study provides helpful evidence of how the size structure of fish assemblages might respond to changes in the thermal regime of lakes in consequence of future climate change (Gardner *et al.*, 2011; Jeppesen *et al.*, 2010). One can expect a decrease in large-sized individuals and a shift towards dominance of small-sized fish with increasing lake temperature.

## 2.5 ACKNOWLEDGMENTS

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