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Relationships between environmental variables and zooplankton subfossils in the surface sediments of 36 shallow coastal brackish lakes with special emphasis on the role of fish

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Abstract

Subfossil zooplankton assemblages (Cladocera 22 taxa, Rotifera 1 taxon) were identified from the surface sediments of 36 shallow (median depth = 0.7 m) Danish coastal brackish lakes differing in epilimnic salinity (SAL, range 0.2–17.4‰), summer-mean total phosphorus (TP, 27–327 μg l^{-1}) and total nitrogen (TN, 0.850–2.629 mg l^{-1}), as well as in submerged macrophyte coverage and planktivorous fish density (PL-CPUE). Cladoceran species richness declined significantly with increasing SAL, TP and TN, while no significant correlation was found to either PL-CPUE, macrophyte coverage or lake surface area. Bonferroni-adjusted forward selection within canonical correspondence analysis (CCA) showed that 22.1% of the variation in zooplankton data was explained by PL-CPUE, SAL and TP uniquely; each variable explaining an almost equally significant amount of variation in the zooplankton data. Predictive models to infer PL-CPUE, SAL and TP were developed using variance weighted-averaging (WA) procedures. Almost similar values of boot-strapped coefficient of determination ($r^2_{\text{boot-strapped}}$ 0.22–0.38) were produced by the WA inference models of PL-CPUE, SAL and TP, while the inference models of TP produced the lowest boot-strapped root-mean-squared-error of prediction (RMSEP$_{\text{boot-strapped}}$ 0.29–0.36 log(TP + 1), μg l^{-1}). Yet, zooplankton TP and SAL optima (WA) were strongly correlated (r^2 = 0.46), while PL-CPUE optima (WA) were independent of both TP and SAL optima, indicating that only the PL-CPUE inference models are suitable for making reconstructions.

Introduction

Salinity is usually considered an important ecological determinant for zooplankton living in saline and brackish lakes. In general, species richness and diversity decrease with increasing salinity over a broad salinity range (Moore 1952; Remane and Schlieper 1971; Hammer 1986; Williams et al. 1990; Frey 1993; Greenwald and Hurlbert 1993; Timms 1993; Jeppesen et al. 1994; Williams 1998; Bos et al. 1999), mainly due to physiological stress resulting from limited osmoregulative capacities (Aladin 1991; Frey 1993) affecting feeding rate, growth, body size, life span, parthenogenetic reproduction and survival (Achthankutty et al. 2000). However, several authors have suggested that species distribution may be determined by other factors in addition to salinity, as non-significant relationships between salinity and species composition within intermediate salinity ranges have been observed in saline lakes (Timms 1983; Williams et al. 1990; Green 1993; Williams 1998; Wolfram et al. 1999; Boronat et al. 2001). Exact information on the importance of ecological factors, especially biotic variables (e.g., fish predation and macrophyte coverage), has yet to be obtained. Contemporary studies indicate that the predation pressure by planktivorous fish and invertebrates on zooplankton is higher in brackish lakes compared to freshwater lakes, partly because of the lack of refuge possibilities within the submerged vegetation, even when macrophytes are abundant, and partly because of a high abundance of such predators (Moss 1994; Jeppesen et al. 1997; Søndergaard et al. 2000). Yet, for the saline lakes, the extent to which zooplankton community structure is affected by various ecological variables and their interactions in brackish lakes is still unclear, which in part is attributed to lack of contemporary data (Jeppesen et al. 1997; Amsinck et al. 2003).

Recently, relationships between zooplankton community structure and a number of environmental variables have been established for freshwater lakes (Hann et al. 1994; Jeppesen et al. 1996, 2002a, 2003; Lotter et al. 1997; Brodersen et al. 1998; Korhola et al. 2000) and saline lakes (Bos et al. 1999), based on zooplankton subfossils deposited in surficial sediments and contemporary, measured environmental variables. A similar approach may provide insight into the impact of the environmental factors on zooplankton species composition in brackish lakes.

In the present study, zooplankton (Cladocera, Rotifera) species composition and richness are related to contemporary ecological variables of 36 Danish coastal brackish lakes (Figure 1) within a salinity range of 0.2–17.4‰. Zooplankton were recovered as subfossils from surface sediments along with measurements of environmental variables including salinity, total phosphorus, total nitrogen, coverage of submerged macrophytes and planktivorous fish density. Multivariate statistical analyses were used to examine patterns of species composition and to identify which environmental variables accounted for most variation observed within the zooplankton species distribution. Finally, predictive models to infer planktivorous fish density, salinity and total phosphorus were developed using variance weighted-averaging (WA) procedures.

Materials and methods

Environmental variables

Lake water total phosphorus (TP) was determined as molybdate reactive phosphorus (Murphy and Riley 1972) following persulphate digestion (Koroleff 1970), and total nitrogen (TN) as the sum of ammonia, nitrite and nitrate after potassium persulphate digestion (Solórzano and Sharp 1980) using the phanate method (APHA 1985). Salinity (SAL) was measured on site using a portable YSI 30. Samples for water chemistry analyses were taken during summer (May–October) between 1996 and 2000 in 15 of the study lakes, while in the remaining lakes sampling was undertaken only once simultaneously with the surface sediment sampling during summer and autumn 1998–1999.

The composition and relative abundance of the planktivorous fish were determined by standardised test-fishing (Mortensen et al. 1991) with multiple mesh size gill nets (6.25, 8, 10, 12.5, 16.5, 22, 25, 30, 33, 38, 43, 50, 60, 75 mm), the length and depth of each section being 3 and 1.5 m, respectively. In lakes with depths <1.5 m, the surplus depth of the net was shortened to prevent entanglement. Depending on lake size, between 1 and 54 nets were used. The nets were set late in the afternoon and retrieved the next morning (ca 18 h). The abundance of planktivorous fish was calculated as mean catch per net and expressed as catch per unit effort (PL-CPUE). The most frequently occurring planktivorous fish were roach (*Rutilus rutilus*), 3- and 9-spined sticklebacks (*Gasterosteus aculeatus*, *Pungitius pungitius*), smelt (*Osmerus eperlanus*) and ruffe (*Gymnocephalus cernuus*), whereas rudd (*Scardinius erythrophtalmus*), bream (*Abramis brama*), tench (*Tinca tinca*), whitefish (*Coregonus lavaretus*) and crucian carp (*Carassius carassius*) were less common. The fishing was conducted once in each lake in late summer between 1991 and 1998. To determine the coverage of submerged macrophytes, the lake area was divided into subareas along transects according to Moeslund et al. (1996) and Søndergaard et al. (1999). Within each subarea the following cate-

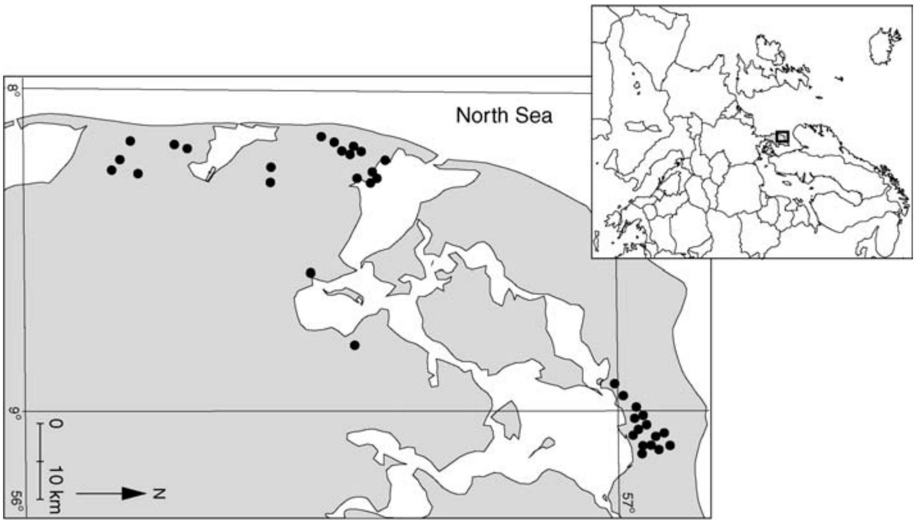

Figure 1. Geographical position of the 36 study lakes.

gories of coverage were used: < 1%, 1–5%, 5–25%, 50–75%, 75–100%. The coverage for the whole lake (MACRO) was calculated according to Jeppesen et al. (1996). The macrophyte studies were conducted between 1993 and 2000. Data on the planktivorous fish stock and macrophyte coverage of 24 study lakes were obtained from technical reports elaborated by the Danish Counties using similar methods as described above.

Subfossil remains

From each lake, five surface sediment (0–1 cm) samples were recovered from the deepest part of the lake using a Kajak corer (internal diameter: 5.2 cm). The sediment samples were pooled for each lake and kept frozen (−18 °C) prior to analysis for cladoceran and rotifer remains. Subfossil remains were extracted from approximately 15 g (wet weight) homogenised sediment. The subsamples were boiled in 30 ml 10% KOH for 20 min and subsequently kept cold (4 °C) for no longer than 2 weeks until counting. Prior to analyses, the samples were exposed to ultrasound (10 s, amplitude 50) and afterwards filtered manually. Remains > 80 μm were identified using a stereomicroscope (100×, Leica MZ12) and an inverted light microscope (320×, Leitz Labovert FS). To facilitate the counting, the remains were divided into two size fractions: > 140 and 80–140 μm. Remains > 140 μm were all counted except for *Chydorus sphaericus*, which was subsampled due to high abundance. Remains in the 80–140 μm size fraction were all subsampled. For identification, the keys of Frey (1959), Margaritora (1985), Hann (1990) and Røen (1995) were used. Counts of exoskeletal remains were adjusted to number of organisms (i.e., whole carapace divided by one, half carapace divided by two). As the different fragments within the Cladocera suborder are unequally preserved, only the most abundant and the most representative fragment of a species was used for data analysis.

Statistical analyses

The full data set included all 36 lakes, five environmental variables (PL-CPUE, SAL, TP, TN, MACRO), 22 cladoceran taxa and 1 rotifer taxon. Data analyses were also conducted on subsets of taxa grouped into two habitat preferences, namely pelagic taxa (4 cladoceran, 1 rotifer) and macrophyte and macrophyte-sediment associated taxa (13 cladoceran), according to Røen (1995) and Hann (1990). Prior to statistical analyses, all environmental variables were $\log(x + 1)$ transformed to stabilise variance and circumvent zero values of the variables. Before transformation, TP and TN were expressed as $\mu g\ l^{-1}$ and SAL as $mg\ l^{-1}$ to minimise the effect of the addition of 1 by log transformation. Subfossil remains were expressed as relative weighted no. for each individual taxon within the 36 lakes to avoid bias towards better preserved taxa in the sediment.

Linear regression

Linear regression (t-test) and significance adjusted by Bonferroni corrected Type 1 error ($\alpha_{\text{corrected}} = 0.05/5 = 0.01$) was performed on all combinations of the measured environmental variables in order to determine their independence. In addition, linear regression was performed to investigate the influence of the environmental variables on the species richness expressed as absolute no. of taxa.

Ordinations

Initial exploratory data analyses were performed using detrended correspondence analysis (DCA) to estimate the species gradient length, with values of axis 1 gradient length >2 standard deviations (SD) indicating that most species respond unimodally to underlying ecological gradients (ter Braak 1995). Correspondence analysis (CA) was used to determine the main directions of variance in the species data among the lakes and to estimate the full variance in species composition across the calibration set. Canonical correspondence analyses (CCA) were performed to examine the relationships between the zooplankton and environmental variables. Site and species scores from CA were linearly correlated to those of the CCA to examine if the major directions of variation in the species data were captured by the measured environmental variables. Tests of significance of the ordination axes were performed by Monto Carlo permutations (999 iterations) by specifying the first, second and third CCA axes, respectively, as covariables (ter Braak and Smilauer 1998). The significance of the forward selected environmental variables under the CCA procedures was tested by Monto Carlo permutations (999 iterations). As the forward selection procedure with a fixed p value is too liberal (ter Braak and Smilauer 1998), adjustment by Bonferroni corrected Type 1 error ($\alpha_{\text{corrected}} = 0.05/5 = 0.01$) was performed. A series of partial CCA ordinations including only the significant environmental variables was run to examine the contribution of explanatory power to the variance in species data by each single variable. The partial CCAs were run with each of the significant variables as an active variable and the other significant variables as co-variables according to Borcard et al. (1992). Single-variable CCAs were performed and the ratio of the first constrained eigenvalue (λ_1) to the second unconstrained eigenvalue (λ_2) was recorded, indicating the viability of constructing an inference model (Kingston et al. 1992). Moreover, single-variable detrended CCAs (DCCA) were performed to explore the species turnover along the individual environmental gradients and thereby determine the potential of applying unimodal-based inference models such as the weighted-averaging (WA) models. All ordination analyses were performed using CANOCO version 4.0 (ter Braak and Smilauer 1998) with no transformation or no down weighting of species data.

Inference models

Quantitative inference models were developed using weighted-averaging (WA) and weighted-averaging with tolerance down weighting (WA (tol)) using the program WACALIB version 3.3 (Line et al. 1994). Both inverse and classical deshrinking regressions of weighted averaging estimates were applied to compensate for the shrinking of estimated ranges of the environmental variable under the procedures of WA regression and WA calibration. In addition, inference models were developed using weighted-averaging partial-least-partial-squares regression (WA-PLS) by the program WA-PLS version 1.51 (ter Braak and Juggins 1993). The optimal number of components to include in the WA-PLS model was assessed by leave-one-out-jack-knifing permutations test (9999 iterations). Models with a higher component number were selected if 5% improvement of the root-mean-squared-error of prediction (RMSEP) could be obtained (Birks 1998). The statistical performance of the inference models was assessed by the coefficient of determination (r^2) between observed and predicted values of the environmental variable, the distribution of residuals (observed value – predicted value) and by RMSEP. Predicted values and RMSEP were obtained by bootstrapping for WA and WA(tol) models and by jack-knife-leave-one-out for WA-PLS models as recommended by Birks (1995). The inference models of TP, SAL and PL-CPUE were based on all zooplankton taxa identified ($n = 23$ taxa). In addition, models of PL-CPUE based on only pelagic taxa ($n = 5$ taxa) were also produced. Optima of TP, SAL and PL-CPUE for each zooplankton taxon were calculated using the weighted averaging method (WA) in the program WACALIB version 3.3 (Line et al. 1994).

Results

The 36 lakes were shallow (0.2–2.9 m) and covered a wide range of surface areas, nutrient concentrations, salinities, fish and plant abundances. However, the majority of the lakes were relatively small, slightly eutrophic with low salinity, low PL-CPUE and moderate MACRO (Table 1).

Ordinations

For the full dataset ($n = 23$ taxa), DCA showed a gradient length > 3 SD (Table 2), suggesting use of unimodal ordination such as CA and CCA (ter Braak and Prentice 1988; ter Braak 1990; Birks 1995). The eigenvalues of the first and the second DCA ordination axes ($\lambda_1 = 0.559$, $\lambda_2 = 0.331$) explained 29% of the cumulative variation in species data. The CCA resulted in two significant axes ($p \leq 0.005$) whose eigenvalues ($\lambda_1 = 0.381$, $\lambda_2 = 0.275$, total inertia = 1.032) were slightly lower than those of CA ($\lambda_1 = 0.559$, $\lambda_2 = 0.412$, Total inertia = 3.067). The correlation between the CA and CCA site and species scores for axis one ($r^2 = 0.87$, 0.76) was high but somewhat lower for axis two ($r^2 = 0.27$, 0.41), indicating that the main direction of variation in the data set was captured by the measured variables, especially on axis one. Bonferroni-adjusted forward selection in CCA showed that PL-CPUE, SAL and TP were the minimum set of significant environmental variables that could explain the greatest amount of variation in the data set. Decomposition of the species variation by partial CCA ordinations showed that 22.1% of the species variance was explained by the three significant variables uniquely, the explanatory power of PL-CPUE being only slightly higher compared to those of SAL and TP (Table 2). Single-variable DCCA for PL-CPUE and SAL showed lengths of gradient of ordination axis one > 2 SD (Table 3), indicating mainly unimodal species-environmental relationships. Single-variable CCA showed that PL-CPUE had higher λ_1/λ_2 ratios (Table 3), suggesting that PL-CPUE was the more suitable candidate for development of transfer functions.

For both subsets of species data, DCA showed gradient lengths > 3 SD (Table 2) with eigenvalues of the first and the second DCA ordination axes explaining 59% and 36% of the cumulative variation in species data for the subset including pelagic taxa ($\lambda_1 = 0.939$, $\lambda_2 = 0.183$) and the subset including macrophyte and macrophyte-sediment associated taxa ($\lambda_1 = 0.594$, $\lambda_2 = 0.275$), respectively. Bonferroni-adjusted forward selection and variance partitioning identified PL-CPUE, TP and macrophytes significant for the pelagic taxa, while PL-CPUE and SAL were found significant for the macrophyte and macrophyte-sediment associated taxa (Table 2). For all significant environmental variables, single-variable DCCA showed gradient lengths of the first ordination axis < 2 SD (range: 0.870–1.939), suggesting that unimodal-based WA models may be less suitable to apply for the subsets.

The species distribution in the CCA ordination (Figure 2) showed that among the pelagic species,

Table 1. Morphometric, chemical and biological data of the 36 brackish lakes

	Mean	Median	Minimum	Maximum
Surface lake area (ha)	171	34	0.4	1048
Mean depth (m)	0.8	0.7	0.2	2.9
SAL (‰)	2.4	0.9	0.2	17.4
TP (mg l^{-1})	0.122	0.079	0.027	0.327
TN (mg l^{-1})	1.929	1.800	0.850	2.629
MACRO (%)	43	33	0	100
PL-CPUE (no. net^{-1})	38	18	0	242
Piscivorous fish (no. net^{-1})	11	3	0	62

the large-sized *Ctenodaphnia* spp. appeared to prefer conditions with high MACRO, low PL-CPUE and high TN in contrast to the smaller body-sized *Daphnia* spp., *B. coregoni* and *B. longirostris*. Pelagic species showed less variation over the range of SAL and TP, with most species optima found near mean values.

Similarly, the macrophyte-associated species *S. crystallina* and *Simocephalus* spp. showed species optima close to mean values of SAL and TP, while *A. harpae*, *G. testudinaria*, *A. nana* and *E. lamellatus* seemed to prefer conditions with low SAL and TP. The large-sized species *E. lamellatus* and *Simocephalus* spp. seemed to prefer higher MACRO and lower PL-CPUE compared to the remaining small-sized macrophyte-associated taxa with the exception of *A. nana*. High salinity preference was shown by *O. tenuicaudis*, the sediment-associated *L. acanthocercoides*, *L. leydigi* and the pelagic rotifer *Brachionus* spp., which are all located to the upper right of the ordination plot. Species with less specific habitat adaptations, such as the macrophyte-sediment associated species, are positioned close to the centre of the ordination plot and thus in between the macrophyte-associated species and the truly sediment-associated species.

Table 2. Results of DCA including all five environmental variables and partial CCA analysis including only significant environmental variables of the full data set and subsets

	Full data set	Pelagic	M and M/S
No. of taxa	23	5	13
DCA (length of gradient)	3.098	3.182	3.533
Environmental variable (% variation explained)			
PL-CPUE	9.7	22.0	10.8
SAL	6.6		10.1
TP	5.8	21.4	
MACRO		10.8	
Total variation explained (%)	22.1	54.2	20.9

M = macrophyte associated taxa, M/S = macrophyte-sediment associated taxa.

Table 3. Results of single-variable CCA (λ_1/λ_2) and single-variable DCCA (length of gradient of 1 ordination axis) on significant environmental variables of the full data set including all taxa identified

Environmental variable	λ_1/λ_2	DCCA gradient length of 1 axis (SD)
PL-CPUE	0.62	2.406
SAL	0.49	2.144
TP	0.48	1.768

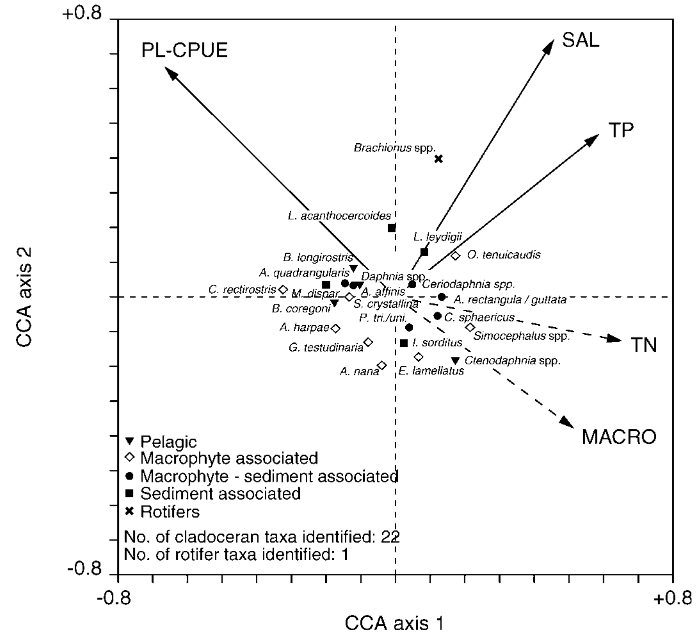

Figure 2. CCA ordination plot of 23 taxa identified in 36 lake surface-sediment samples. Solid arrows indicate significant variables determined by Bonferroni-adjusted forward selection ($p < 0.01$), while dotted arrows indicate non-significant variables. Eigenvalue $\lambda = 0.381$ (CCA axis 1) and $\lambda = 0.275$ (CCA axis 2).

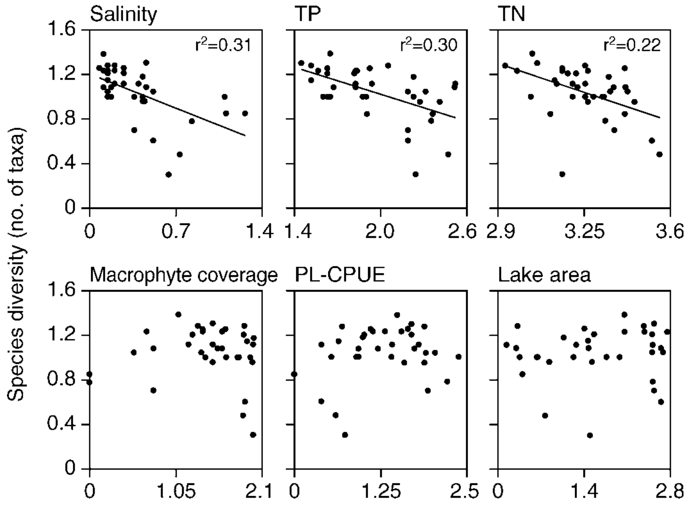

Figure 3. Relationships between species richness (absolute no. of identified taxa) and measured environmental variables. All variables including species richness are log(x + 1) transformed.

Linear regression

Regression analysis revealed that only PL-CPUE could be considered as an independent variable. TP was positively correlated with both SAL ($p < 0.0003$, $r^2 = 0.32$) and TN ($p < 0.0001$, $r^2 = 0.42$), while SAL was slightly negatively correlated to MACRO ($p < 0.0096$, $r^2 = 0.18$). Species richness was significantly negatively correlated to SAL ($p < 0.0004$, $r^2 = 0.31$), TP ($p < 0.0006$, $r^2 = 0.30$) and TN ($p < 0.0042$, $r^2 = 0.22$), while no significant correlation was found to either PL-CPUE ($p > 0.13$, $r^2 = 0.07$), MACRO ($p > 0.76$, $r^2 = 0.003$) or surface lake area ($p > 0.35$, $r^2 = 0.03$); (Figure 3).

Inference models for PL-CPUE, SAL and TP

Second component WA-PLS models based on the full data set ($n = 23$ taxa) did not contribute to a 5% improvement of RMSEP compared to one component WA-PLS models. As the one component WA-PLS model is identical with the WA model with inverse deshrinking (ter Braak and Juggins 1993), only the results of the latter model are shown and described here. WA inference models of PL-CPUE, SAL and TP based on the full data set produced almost similar values of boot-strapped coefficient of determination ($r^2_{\text{boot-strapped}}$ 0.22–0.38) (Table 4). WA models based on inverse deshrinking regression produced lower RMSEP than classical deshrinking regression models, with almost similar performance of WA and WA(tol) models (Table 4). However, all WA models based on inverse deshrinking regression produced a significant bias in residuals distribution with a tendency to overestimation at low ranges and underestimation within high ranges, whereas no significant bias was found by models based on classical deshrinking ($p > 0.05$) (Table 4, Figure 4). The first error component (RMSE_{si1}) of the WA models, expect models for inference of SAL based on classical regression, indicated that the calibration set is adequate to yield reliable estimates of taxon optima as required in WA, but not large enough for reliable estimation of all taxon tolerances used in WA(tol), as the RMSE_{si1} was small for WA, but large for WA(tol) (Table 4); (Birks 1994). The second error component (RMSE_{s2}) suggested considerable natural variation in the zooplankton assemblages at a given value of PL-CPUE, SAL or TP and that similar zooplankton assemblages can occur at different values of PL-CPUE, SAL or TP, RMSE_{s2} being larger than RMSE_{si1} (Table 4); (Birks 1994). WA models of TP produced the lowest boot-strapped root-mean-squared-error of prediction ($\text{RMSEP}_{\text{boot-strapped}}$0.29–0.36 log(TP + 1), μg l^{-1}) compared to the models of SAL and PL-CPUE (Table 4). The zooplankton WA optima of TP were significantly correlated to those of SAL

($r^2 = 0.46$), while zooplankton WA optima of PL-CPUE were independent of both SAL and TP (Figure 5).

WA inference models of PL-CPUE based on only the pelagic taxa ($n = 5$ taxa) produced low apparent r^2 ($r^2 = 0.20$) and high RMSE (RMSEP = 0.589 log (PL-CPUE + 1), fish net^{-1}) under bootstrapping. Moreover, models for inference of PL-CPUE including all identified taxa ($n = 23$ taxa) were evenly distributed along the regression line (Figure 4A and B), whereas models of the pelagic taxa were unevenly distributed. Due to similarity of the WA models based on pelagic taxa, only the WA model with inverse deshrinking is shown (Figure 4C).

Discussion

The present study indicates that salinity and TP are significant variables influencing zooplankton community structure and cladoceran species richness in shallow brackish lakes. At high salinity and nutrient levels, *L. acanthocercoides*, *L. leydigi* and *O. tenuicaudis* and the rotifer *Brachionus* spp. had the greatest abundance in the surface sediments. At intermediate salinity levels *Ceriodaphnia* spp., *A. rectangula*/*gutta*, *C. sphaericus* and *B. longirostris* were more abundant, while the remaining species mainly occurred in lakes with low salinity and low nutrient levels (Figure 2). This agrees with previous studies in which relatively high salinity tolerance levels in athalassic saline lakes were observed for *L. acanthocercoides* (< 3.5‰), *O. tenuicaudis* (< 6‰), *B. longirostris* (< 8‰), *A. rectangula* (< 12‰) and *C. sphaericus* (56‰) (Löffler 1961; Hammer 1986; Aladin 1991; Wolfram et al. 1999). In brackish Danish lakes, *B. longirostris* was the exclusively dominant cladoceran in the pelagic zone in the 7–16‰ salinity range (Jeppesen et al. 1994). In the Baltic Sea, *B. longirostris* and *C. sphaericus* occurred at salinities up to 6‰ and 10.5‰, respectively, while the remaining chydorid species occurred at salinities up to 3–5‰ (Flössner 1972). Moreover, enclosure experiments conducted along a salinity gradient (0.5–12‰) in the Danish brackish lake Kogleakssøen showed a pronounced shift from cladoceran to rotifer (including *Brachionus* spp.) dominance at salinities > 8‰ (Jeppesen et al. 2002b). Brodersen et al. (1998) found high TP optima (near 140 μg l^{-1}) for *L. leydigi*, *A. rectan-*

Table 4. Summary statistics for inference models for reconstruction of PL-CPUE, SAL and TP based on the entire zooplankton data set ($n = 23$ taxa) and 36 brackish lakes

Environmental variable	Model	r^2_{apparent}	$r^2_{\text{boot-strapped}}$	RMSEP	RMSE$_{\text{si1}}$	RMSE$_{\text{s2}}$	$r^2_{\text{residuals}}$
PL-CPUE	Inverse deshrinking						
	WA	0.535	0.351	0.521	0.189	0.486	0.465
	WA(tol)	0.561	0.300	0.557	0.230	0.507	0.439
	Classical deshrinking						
	WA	0.535	0.380	0.666	0.339	0.573	n.s.
	WA(tol)	0.561	0.325	0.728	0.423	0.592	n.s.
SAL	Inverse deshrinking						
	WA	0.387	0.222	0.449	0.139	0.427	0.613
	WA(tol)	0.513	0.334	0.428	0.158	0.398	0.487
	Classical deshrinking						
	WA	0.387	0.285	0.621	0.330	0.526	n.s.
	WA(tol)	0.513	0.366	0.550	0.305	0.457	n.s.
TP	Inverse deshrinking						
	WA	0.525	0.315	0.287	0.108	0.266	0.475
	WA(tol)	0.563	0.314	0.294	0.124	0.266	0.437
	Classical deshrinking						
	WA	0.525	0.362	0.364	0.188	0.311	n.s.
	WA(tol)	0.563	0.361	0.364	0.209	0.297	n.s.

Models developed using the program WACALIB version 3.3 (Line et al. 1994). WA = weighted-averaging, WA(tol) weighted-averaging with tolerance down weighting. Permutation test by bootstrapping with 999 iterations. Units of RMSE and RMSEP = log (PL-CPUE + 1), fish net^{-1}. n.s. = non-significant.

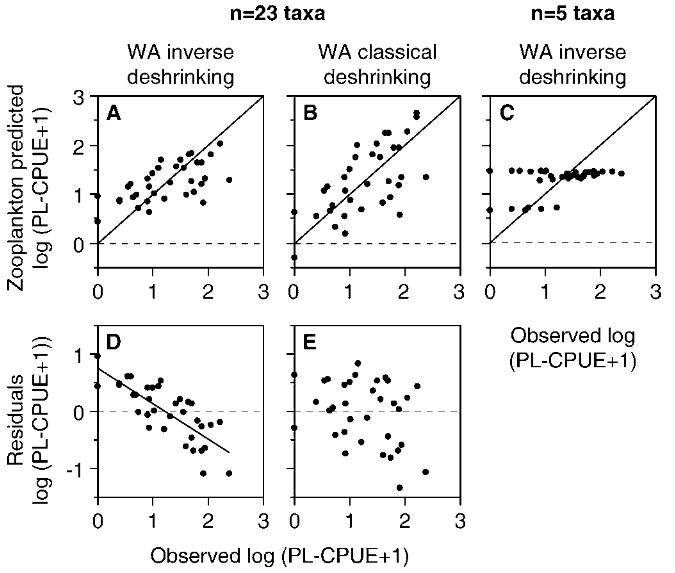

Figure 4. Predicted PL-CPUE and associated residual distribution against observed PL-CPUE: (4A, 4D) WA model based on inverse deshrinking regression, (4B, 4E) the WA model based on classical deshrinking regression based on the full data set ($n = 23$ taxa), (4C) WA model based on inverse deshrinking regression based on the pelagic taxa ($n = 5$). Predicted values calculated by bootstrapping for the WA models (999 iterations). Line 1:1 drawn in 4A, 4B and 4C, while regression line inserted in 4D. Data based on 36 brackish lakes.

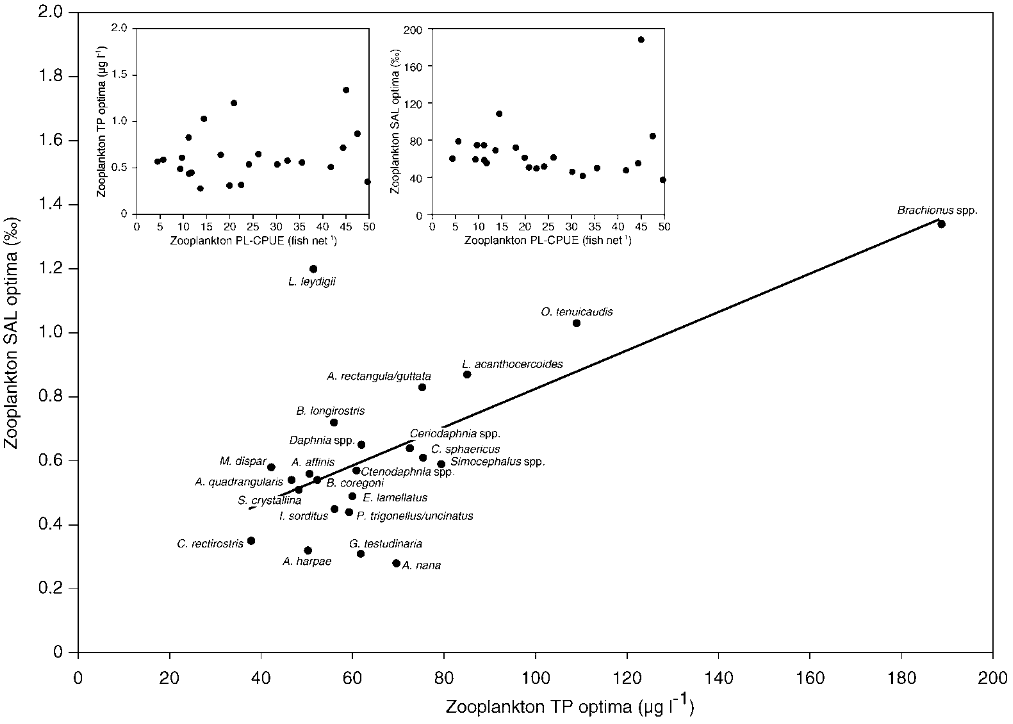

Figure 5. Relationship between zooplankton weighted average (WA) TP optima and WA SAL optima. Inserted diagrams: Relationship between zooplankton WA PL-CPUE optima and WA TP and SAL optima, respectively.

gula and *C. sphaericus*, intermediate TP (60–140 μg l^{-1}) for *A. affinis*, *E. lamellatus*, *A. harpae*, *M. dispar* and *G. testudinaria* and low TP (<40 μg l^{-1}) for *A. nana* in a screening of surficial sediments of 32 Danish freshwater lakes, which is consistent with the present species distribution along the TP gradient (Figure 2). In accordance, other studies (e.g., Whiteside 1970; Frey 1986; Hofmann 1996) have reported high tolerances of *C. sphaericus* and *A. rectangula* to nutrient-rich conditions in freshwater lakes, findings that have recently been confirmed by Eyto et al. (2003) in a study of the cladoceran distribution in 66 shallow freshwater lakes across Europe.

A clear trend of declining species richness with increasing levels of salinity was found (Figure 3). This is in agreement with other observations from saline lakes (Hammer et al. 1990; Frey 1993; Bos et al. 1999; Boronat et al. 2001). Declining species richness was also found with increasing nutrient levels (Figure 3). However, in contrast to salinity, to which the response of species richness can be explained by differences in osmoregulative capacities among the cladoceran taxa (Aladin 1991; Frey 1993; Aladin and Potts 1995), the response to nutrients is less clear. In freshwater lakes, declining cladoceran richness with increasing nutrient enrichment is generally considered to be linked with reduced habitat diversity by loss of macrophytes (Jeppesen et al. 2001). However, in the present study macrophyte coverage was independent of both TP and TN, which is consistent with studies undertaken in other European brackish lakes (Moss 1994; Jeppesen et al. 1994). Yet, species richness showed no relationship with macrophyte coverage (Figure 3). Moreover, enclosure studies performed along a nutrient gradient with a constant salinity level (0.5‰) showed no decrease in cladoceran species richness with increasing nutrient levels (Jeppesen et al. 2002b). The observed decline in species richness with increasing TP may be caused by an indirect impact by salinity on cladocerans, as TP and salinity were found to be correlated.

The density of planktivorous fish seemed to play a major role for the entire cladoceran community structure (Table 2). In lakes with intermediate to high fish predation pressure, small-sized cladocerans dominated, *B. longirostris*, *B. coregoni*, *M. dispar*, *A. quadrangularis* and *A. affinis* being the most abundant species, while large-sized cladocerans such as *Ctenodaphnia* spp., *E. lamellatus* and *Simocephalus* spp. apparently preferred lakes with low predation pressure (Figure 2), which indicates that size-selective fish predation, as known from freshwater lakes (Kitchell and Kitchell 1980), also plays an important structuring role for the cladoceran size composition in brackish lakes, as earlier suggested from analysis of contemporary data (Jeppesen et al. 1994, 1997).

Coverage of macrophytes seemed to be of only minor importance in shaping the cladoceran species community structure (Table 2). This agrees with previous studies in brackish lakes, in which a high predation pressure on cladocerans by planktivorous fish was found even at high submerged macrophyte abundances, implying that the importance of submerged macrophytes as refuges for cladocerans against fish predation is limited in brackish lakes (Jeppesen et al. 1994, 1997). The finding that PL-CPUE in all habitat groups examined contributed significantly to the species variation (Table 2) further supports observations of a high predation pressure within macrophyte beds (Moss 1994; Søndergaard et al. 2000). However, it is important to emphasise that of the 36 studied lakes, only 14 had a macrophyte coverage $>45\%$, of which 8 lakes had small surface areas (<11 ha) and low numbers of planktivorous fish (PL-CPUE <9 fish net^{-1}), with the exception of one lake (PL-CPUE: 39 fish net^{-1}). Thus, the low number of lakes with relatively high macrophyte abundances may have contributed to the finding that macrophytes were only of minor importance.

Weighted-averaging models based on all zooplankton taxa identified ($n = 23$ taxa) and developed for inference of TP produced lower RMSEP compared to similar models of PL-CPUE and SAL (Table 4), suggesting that the TP models produce more reliable results. Yet, zooplankton TP and SAL optima (WA) were strongly correlated ($r^2 = 0.46$) (Figure 5), indicating difficulties in pinpointing which of the two variables is the main controlling factor and responsible for the zooplankton distribution. By contrast, zooplankton WA optima of PL-CPUE were independent of both the zooplankton WA optima of SAL and TP (Figure 5), making the PL-CPUE inference models more suitable for reconstructions.

Among the WA models developed for inference of PL-CPUE, the models based on all taxa identified ($n = 23$ taxa) produced a more reliable inference than models based solely on pelagic taxa ($n = 5$), as only the former produced an even distribution between inferred and observed PL-CPUE values (Figure 4A and B). Among the weighted averaging models using 23 taxa, the simple WA-model based on inverse deshrinking produced the lowest RMSEP (Table 4). However, this model also produced a distinct bias in residual distribution Figure 4D), indicating lower applicability of PL-CPUE inference, especially in the lower (ca. < 5 fish net^{-1}) and upper (ca. > 100 fish net^{-1}) end of the PL-CPUE gradient. The simple WA-model produced comparable estimates of r^2 and RMSE to similar models developed on 6 pelagic zooplankton taxa with significant relationships to PL-CPUE in freshwater lakes (WA: $r^2_{\text{apparent}} = 0.60$, RMSE $= 0.64$, WA(tol): $r^2_{\text{apparent}} = 0.56$, RMSE $= 0.54$); (Jeppesen et al. 1996). By contrast, models including only pelagic taxa for the brackish lakes produced lower estimates of r^2_{apparent} and higher estimates of RMSE, which may indicate potential problems when implementing models based on only few taxa, as the different taxa show different salinity tolerances. By way of example, the small body-sized *B. longirostris* has a higher salinity tolerance compared to the large body-sized *Ctenodaphnia* spp. Thus, under conditions of relatively high salinity impact, absence of *Ctenodaphnia* spp. and presence of *B. longirostris* may be more directly linked to salinity than to size selective fish predation. As for freshwater lakes (Jeppesen et al. 1996), the relationship between zooplankton predicted and observed PL-CPUE in brackish lakes is less precise in comparison with cladoceran based inference models of TP (Brodersen et al. 1998; Bos and Cumming 2003) or cladoceran and anostracan based inference models of salinity (Bos et al. 1999), as well as with diatom based inference models of TP and salinity (Hall and Smol 1992; Bennion et al. 1996; Reed 1998; Ryves et al. 2002). This is to be expected, as estimations of fish abundances in the field are prone to several uncertainties (Jeppesen et al. 1996). The PL-CPUE inference models will most likely be strengthened, and their application further improved, by the addition of more lakes in the regime of high salinity and high PL-CPUE, as the median values of both salinity (0.9‰) and PL-CPUE (18 fish net^{-1}) in the calibration lakes are relatively low. An expansion of the existing data set with more lakes may furthermore allow use of linear-based analogue statistics, minimising the undesirable edge effects inherent in uni-model based WA methods (Birks 1998). Moreover, development of data sets including brackish lakes along a gradient of TP at constant salinity regimes and vice visa will most likely contribute to increased insight into how the biological structure is affected by the environmental variables individually, and thus circumvent the problems of correlation found in this study.

In summary, the species composition of zooplankton subfossils in the surface sediments of 36 coastal shallow brackish lakes showed significant relationships with planktivorous fish density, salinity and total phosphorus, whereas submerged coverage of macrophytes was found to be of less importance. Cladoceran species richness was negatively related to salinity and nutrients (TP, TN). Weighted averaging models for the reconstruction of planktivorous fish density, salinity and total phosphorus were established. Yet only the former model was suitable for reconstructions as strong correlation was found between the zooplankton WA optima of total phosphorus and salinity.

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