

## Factors influencing zooplankton size structure at contrasting temperatures in coastal shallow lakes: Implications for effects of climate change

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### Abstract

We assessed the importance of temperature, salinity, and predation for the size structure of zooplankton and provided insight into the future ecological structure and function of shallow lakes in a warmer climate. Artificial plants were introduced in eight comparable coastal shallow brackish lakes located at two contrasting temperatures: cold-temperate and Mediterranean climate region. Zooplankton, fish, and macroinvertebrates were sampled within the plants and at open-water habitats. The fish communities of these brackish lakes were characterized by small-sized individuals, highly associated with submerged plants. Overall, higher densities of small planktivorous fish were recorded in the Mediterranean compared to the cold-temperate region, likely reflecting temperature-related differences as have been observed in freshwater lakes. Our results suggest that fish predation is the major control of zooplankton size structure in brackish lakes, since fish density was related to a decrease in mean body size and density of zooplankton and this was reflected in a unimodal shaped biomass-size spectrum with dominance of small sizes and low size diversity. Salinity might play a more indirect role by shaping zooplankton communities toward more salt-tolerant species. In a global-warming perspective, these results suggest that changes in the trophic structure of shallow lakes in temperate regions might be expected as a result of the warmer temperatures and the potentially associated increases in salinity. The decrease in the density of large-bodied zooplankton might reduce the grazing on phytoplankton and thus the chances of maintaining the clear water state in these ecosystems.

The structure and the functioning of shallow lakes might be affected by climate warming in several ways, either directly by the higher temperature or indirectly by changes in salinity and nutrient loading (Jeppesen et al. 2009). A higher temperature has been associated to a shift in fish communities toward smaller sizes and higher densities due to enhanced and earlier reproduction as well as faster growth rate and reduced longevity (Blanck and Lammouroux 2007; Jeppesen et al. 2010). The resulting increase in fish predation may reduce the chances of maintaining the clear water state by decreasing the density of large-bodied zooplankton and thus the control on phytoplankton (Petchey et al. 1999; Moss et al. 2004; Meerhoff et al. 2007a). Thus, several studies (Meschiatti et al. 2000; Blanco et al. 2003; Meerhoff et al. 2007b) have shown that warm lakes exhibit a fish community dominated by small-sized specimens, a high degree of omnivorous feeding (Teixeira-de Mello et al. 2009; Jeppesen et al. 2010), and more frequent spawning than in temperate lakes. Small omnivorous fish may aggregate within the vegetation, and the cladoceran zooplankton may therefore not be able to use submerged macrophytes as a daytime refuge to the same extent as in temperate freshwater lakes dominated by larger planktivorous fish

(Meerhoff et al. 2007a). The result is a high fish predation pressure and a decrease in large and more efficient filter-feeding zooplankton, with major implications for the water clarity of warm lakes (Meerhoff et al. 2007b). Accordingly, several studies have shown that warm lakes have higher sensitivity to eutrophication than temperate lakes (Moss et al. 2004; Jeppesen et al. 2007b; 2009). Warming may also directly affect the zooplankton size structure. Experimental studies showed that the body size of a single zooplankton population is inversely correlated with temperature in the absence of size-selective predation (Moore and Folt 1993). However, despite the importance of zooplankton body size for the maintenance of the clear water state, few studies have assessed the factors determining zooplankton size structure in different climates. In addition, most of these studies have focused on the changes in species composition and biomass of zooplankton and, furthermore, they have often neglected rotifers and paid little attention to changes in community size structure. Surprisingly, when comparing the zooplankton size structure in temperate North American and in tropical African deep lakes, Sprules (2008) found little or no difference between climate zones despite differences in zooplankton species composition and fish community structure.

Warmer temperatures may also enhance evaporation processes, leading to an increase in salinity in both

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freshwater and brackish lakes, especially in arid and semiarid climates (Williams 2001). Increased salinization has also been reported as a result of sea-level rise (Schallenberg et al. 2003) and due to enhanced use of freshwater for, for instance, irrigation and industry (Williams 2001). However, the indirect effects of warming in the food-web structure of shallow lakes, such as increased salinity, have been little studied. A recent cross-comparison of coastal brackish lagoons between the cold-temperate and the Mediterranean climate (Bruçet et al. 2009) showed that differences in temperature did not have major effects on zooplankton richness, whereas salinity was the most important factor structuring zooplankton communities in brackish lagoons. This would imply that the indirect effects of climate warming, such as changes in salinity and hydrology, will have larger consequences on brackish lake ecosystems than the increase in temperature per se. Along the salinity gradient a shift occurs from dominance of large and more efficient filter-feeding cladoceran species, such as *Daphnia* spp., at low salinities to dominance of copepods and small cladoceran species, such as *Bosmina* and *Chydorus* spp., at higher salinities (Jeppesen et al. 2007a; Bruçet et al. 2009; Jensen et al. 2010). Such a change in species composition along the salinity gradient may weaken the top-down control on phytoplankton at the higher salinities. Moreover, there is also emerging evidence that fish communities may change along the salinity gradient (Jensen et al. 2010). Recent studies have shown that eutrophic and hypertrophic brackish temperate lakes hold a fish community resembling that of warm lakes, that is, with dominance of small planktivorous fish, such as three-spined stickleback (*Gasterosteus aculeatus*) and nine-spined stickleback (*Pungitius pungitius*), which have more cohorts per year than fish in comparable freshwater lakes (Jeppesen et al. 1994, 2007a) and may predate intensively on the zooplankton (Jakobsen et al. 2003). This would explain why nutrient-rich brackish lakes remain turbid even at high macrophyte coverage (Jeppesen et al. 1994). In addition, pelagic invertebrate predators may be abundant in brackish lakes, notably the shrimp *Neomysis integer* at salinities above 0.5‰ (Irvine et al. 1990; Jeppesen et al. 1994). Thus, in a climate-warming perspective, changes in the community structure of shallow lakes are expected as a combined effect of increased temperature and salinity, although few studies exist yet.

In the present study, we aimed at assessing the importance of salinity and predation (fish and macroinvertebrates) for the zooplankton size structure of coastal shallow lakes in different climate regions and at different habitat complexity. By comparing similar lakes located at contrasting temperatures, we aimed at providing insight into the role of climate change in shallow temperate lake ecosystems. We performed an experiment with artificial plants in eight coastal brackish lakes in cold-temperate (Denmark) and Mediterranean (Spain) regions. We also sampled littoral areas where natural plants were removed and open areas outside the plant-littoral zone. We hypothesized that, owing to the different fish communities and higher fish densities in brackish lakes, macrophytes would not provide an adequate refuge to the zooplankton,

contrasting the findings from temperate freshwater shallow lakes, and hence we expected that fish would affect the shape of the zooplankton size distribution through size-selective predation. We also expected that the trophic structure of brackish lakes would be more similar along a latitude (temperature) gradient than in freshwater lakes, since salinity and planktivorous fish will play a far more important role in the shaping of trophic structure in brackish lakes than will temperature.

To test these hypotheses we used size approaches, particularly the biomass-size spectrum and size diversity. Both biomass-size spectrum and size diversity provide an integrative measure to quantify variations in the structure of zooplankton communities (Kerr and Dickie 2001; Bruçet et al. 2006), and they have been found to shift in relation to predation pressure (Bruçet et al. 2005a; Badosa et al. 2007). Despite that both methods are particularly useful when comparing areas with different taxonomic compositions, they have only been used sparingly as a response variable in experimental field studies.

## Methods

*Experimental design*—The experiment was carried out in four cold-temperate shallow coastal lakes located in the north of Denmark and in four Mediterranean shallow coastal lakes located in the northeast of Spain. Both Spain and Denmark belong to the temperate mesothermal climate region, but they have different climates according to the Köppen Climate Classification System. The region of Spain where the lakes were located (Catalonia) has a semiarid climate characterized by hot and dry summers and cool and wet winters (average temperature 15–16°C, average rainfall 591 mm). Denmark has a moist continental climate with milder summers and colder and somewhat wetter winters (average temperature and rainfall in the region where the lakes were located 7.5–8.1°C and 750–800 mm, respectively). In both regions, we selected shallow lakes with similar total nutrient concentrations and salinities, ranging from 0.3‰ to 3.8‰ during the study period (Table 1). The Spanish lakes under study were originally mouths of different rivers, which were diverted to different locations. The Danish lakes were originally shallow branches of the Limfjord Fiord and were created after damming and draining of the fiord as a result of land reclamation. Although some of them are connected by channels, they serve largely as independent systems due to a low hydraulic loading, since most water from the catchments bypasses the lakes. Thus, although some of them had low salinities during sampling, all lakes under study are transitional environments between land and sea, partially influenced by saline water as a result of their proximity to coastal waters but also substantially influenced by freshwater flows since they are in the vicinity of river mouths and with a mean annual salinity over 0.5‰. The dominant macrophyte species were *Chara aspera* and *Myriophyllum spicatum* in Danish lakes, and in Spanish lakes *Potamogeton pectinatus*. The experiment was conducted in late May and early June in Spain and in July in Denmark.

Artificial plants mimicking submerged plants were introduced in the littoral zone of the lakes following a

Table 1. Main limnological characteristics of the eight study lakes at the time of the experiment. TP, total phosphorus; TN, total nitrogen.

	Denmark				Spain			
	Lund Fjord	Selbjerg	Glombak	Østerild	Salins	Sirvent	Bassa Coll	Ter Vell
Salinity (‰)	0.3	0.5	1.2	3.8	0.4	0.8	1.6	2.2
Temperature (°C)	17.3	16.7	15.8	17.1	23.1	20.2	20.6	21.7
Secchi depth (m)	0.3	0.2	0.5	0.4	0.6	0.8	0.5	0.4
TP (mg L <sup>-1</sup> )	0.10	0.16	0.09	0.08	0.13	0.03	0.17	0.32
TN (mg L <sup>-1</sup> )	1.81	3.64	2.21	2.2	0.44	7.2	1.53	0.42

similar methodology described by Meerhoff et al. (2007a). The plant beds consisted of 1-m-diameter plastic rings with an attached net from which the artificial plants hung (modules). Plants were made of green plastic Christmas tree garlands, which have an architecture resembling that of *Ceratophyllum* or *Myriophyllum* spp. and with a local percentage volume inhabited by plants of 49% (PVI, Canfield et al. 1984; see picture in Meerhoff et al. 2007a). Each module consisted of 100 artificial plants (length 0.75 m), which were held at the surface by two strings attached to two poles. Modules were placed at 0.8 m depth in the littoral zone of the lakes. Before the introduction of the artificial plant beds, natural plants, if present, were removed in ca. 3-m distance around the modules.

We introduced 16 modules per lake: eight modules containing submerged plants (four for nighttime samples and four for daytime samples), and eight modules with only poles and no plants (as a methodological control). The modules were placed 1 month before the sampling in order to allow colonization of the plants by periphyton and invertebrates.

**Sampling and processing**—Depth-integrated water samples were collected with a core sampler from the open water for analysis of total phosphorus and total nitrogen (Grasshoff et al. 1983; APHA 1989). Water transparency was measured with a Secchi disk.

Zooplankton samples were taken from each submerged plant module (hereafter termed “submerged plants”: “S” sites where natural plants, if present, were removed prior to the introduction of the artificial plant beds), from the modules without plants (hereafter “intermediate”: “I” sites where the plants present were removed, and no artificial plants were introduced), and from four “open-water” sites nearby (“O” sites initially without plants and with no modules). In two Danish lakes (Selbjerg and Glombak) there were no true open-water areas near the experimental location. Since sampling in a far zone of the lake would likely result in quite different conditions, we decided to exclude the open-water habitat for these two lakes. We collected zooplankton around midday, “D” and midnight, “N” in order to detect possible diel changes in zooplankton distribution caused by the fish and macroinvertebrate predation pressure (Meerhoff et al. 2007a). We took four open-water samples at random, while submerged plants and intermediate samples were taken during the day in half of the submerged plants and intermediate sites, respectively, and during the night in the other half. Samples were

taken from a boat to minimize sediment resuspension, using a 1-m long core sampler (diameter 6 cm) to collect water from the surface to just above the sediment. The water (10–12 liters) collected from different spots within each module was mixed in a barrel and an 8-liter subsample was filtered through a 50- $\mu$ m mesh filter. Zooplankton was immediately fixed in acid Lugol’s solution (4%).

Fish and free-swimming macroinvertebrate predators were sampled day and night in each module using a cylindrical net (1.1 m in diameter and 1 mm mesh size) attached with strings to two poles and placed over the sediment under each module 24 h before. The net was lifted up quickly by pulling the strings. Additionally, strictly plant-associated macroinvertebrate predators larger than 200  $\mu$ m were sampled by carefully removing three artificial plants from each plant bed. Fish and macroinvertebrates were preserved in 70% ethanol.

We counted (at least 100 individuals of the most abundant zooplankton taxa) and identified (to species level except some Rotifera that were identified to genus) all zooplankton taxa. In order not to miss rare species, we examined the whole sample. Calanoid and cyclopoid copepods were separated into nauplii, copepodites, and adults. The genera *Daphnia*, *Simocephalus*, and *Sida* were classified as large-bodied cladocerans. Biomass dry weight (dry wt) estimations were obtained from the allometric relationship between the weight and the length of the body (Dumont et al. 1975; Botrell et al. 1976; McCauley 1984). For Rotifera, biomass dry weight was calculated by converting biovolume into dry weight (Ruttner-Kolisko 1977; Malley et al. 1989). Rotifera biovolume was estimated from the measurements of the principal diameters of the organisms (Malley et al. 1989). We measured up to 50 individuals of each taxon whenever possible. We counted, measured, and identified fish to species level and categorized them as planktivorous or piscivorous based on Muus and Dahlström (1990), Mittelbach and Persson (1998), and Blanco et al. (2003). Macroinvertebrates were counted and identified to at least family level and classified as potentially predatory or not predatory according to Merritt and Cummins (1996), Tachet et al. (2000), and Monakov (2003). Abundances of macroinvertebrate predators sampled by removing artificial plants (hereafter called “plant-associated macroinvertebrate predators”) were assessed separately from the abundances of macroinvertebrate predators sampled using the nets (hereafter called “free-swimming macroinvertebrate predators”). In addition, we assessed abundances of *Neomysis*, since it has been



reported as an abundant and highly voracious predator of zooplankton, potentially exerting a substantial effect on the whole trophic structure of brackish lakes (Irvine et al. 1990; Jeppesen et al. 1994).

*Size-based approaches*—The size-based approaches focus on the aggregation of the organisms according to their individual body weight regardless of their taxonomy. They have significant advantages over the taxon-based and functional approaches, such as its relation with the metabolism and energy flux through community and its simplicity, since they avoid defining distinct trophic levels or distinguishing between taxonomic groups. The biomass-size spectrum describes how biomass of organisms is distributed along size classes (Kerr and Dickie 2001). It is a useful tool to summarize ecosystem information; however, it is often merely used as a descriptive tool due to the complexity of working with nonlinear distributions characterizing size spectra (Brucet et al. 2005a,b). Size diversity gives a unique value per size distribution, which integrates the amplitude of the size range and the evenness, that is, the relative distribution of sizes along the size range. This simplifies the comparison among samples. Size diversity also has the advantage of an intuitive interpretation of its ecological meaning, since the concept of diversity is well established (Quiroga et al. 2005; Brucet et al. 2006; Quintana et al. 2008). Both methods provide a way of comparing ecosystems that may have quite different species compositions.

*Data analysis*—We used a nested ANOVA to test differences in planktivorous fish and macroinvertebrate predator densities between regions, lakes, habitat, and time. The factors were “region” (two levels); “lake” (four levels), nested within region; “habitat” (two levels, S and I); and “time” (two levels, D and N). The nested ANOVA for the density of plant-associated macroinvertebrate predators had only three factors: region, lake, and time. We prior  $\log_{10}(x + 1)$ -transformed data to fulfill requirements of homoscedasticity and normal distribution of residuals.

We calculated size diversity for each replica following Quintana et al. (2008). The proposed size diversity ( $\mu$ ) is computed based on the Shannon diversity expression adapted for a continuous variable, such as body size. This measure takes the form of an integral involving the probability density function of the size of the individuals described by the following equation:

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

where  $p_x(x)$  is the probability density function of size  $x$ . Nonparametric kernel estimation was used as a probability density function, after data standardization using division of sample data by their geometric mean value. We calculated the average size diversity of the four replicas in each habitat (S, I, O) and for each time (D and N). Since size distribution is usually positively skewed, the mean body size of individuals was estimated using a geometric mean since it is the best estimate of central tendency of positively skewed distributions.

To identify relationships between zooplankton community parameters (density and size) and salinity and density of predators (fish and macroinvertebrate predators) we used multiple regression. As independent variables we used salinity, mean density of planktivorous fish per lake, mean density of planktivorous fish per habitat, mean density of plant-associated macroinvertebrate predators, and mean density of free-swimming macroinvertebrate predators. Response variables in each multiple regression were the following: zooplankton mean body size, zooplankton size diversity, zooplankton total density and total biomass, and the density of each zooplankton group (rotifers, cladocerans, and cyclopoids). All (fish, macroinvertebrates, and zooplankton) densities were calculated as abundances. Error distribution was checked in order to ensure that errors fulfilled the normality and homoscedasticity assumptions. The condition index was never higher than 15 and tolerance never higher than 0.2, suggesting that multicollinearity between predictory variables was low.

We constructed a normalized biomass-size spectrum for each replica by grouping zooplankton individuals into  $\log_2$  size classes, summing up the biomass for each interval, and dividing it by the width in micrograms of that size class (Kerr and Dickie 2001; De Eyto and Irvine 2007). Normalized biomass-size spectrum therefore represents  $\log_2$  (biomass in size class : width of size class) as a function of  $\log_2$  (upper limit of size class). Normalized biomass is roughly equivalent to organism density (individuals per liter) since it represents biomass concentration (micrograms per liter) divided by organism weight (micrograms) (Rodríguez and Mullin 1986; Kerr and Dickie 2001). We developed biomass-size spectra for each habitat (S, I, O) and time (D and N) by calculating the average biomass-size spectrum of the four replicas in each habitat and for each time. We also constructed mean biomass-size spectrum (integrating habitat and time) for each lake.

We used generalized additive modeling (GAM) following De Eyto and Irvine (2007) in the computer package Brodgar version 2.4.3 ([www.brodgar.com](http://www.brodgar.com)) to analyze the zooplankton normalized biomass-size spectra since the size distribution at ecological scale (zooplankton functional group) usually has a nonlinear shape (Brucet et al. 2005a,b). The response variable (“zooplankton normalized biomass”) was modeled using “zooplankton size class,” “density of planktivorous fish” (in captured abundance) and “density of macroinvertebrate predators” (in captured abundance) as the smoothing terms, while lake, habitat, and time were included as nominal variables. An interaction term between lake and habitat and lake and time was included in the model. In order to know whether the observed relationship between the normalized biomass and the size class was the same in all lakes, we included an interaction term between lake and the size class. A significant result in this interaction term indicates that the relationship between the size class and normalized biomass is not the same in the different lakes. Gaussian distribution was selected since it ensured a homogeneous spread of residuals. The optimal degrees of freedom of the smoothing term were found using cross-validation (Wood 2004). Selbjerg (the lake with the highest fish density), interme-

Table 2. Relative abundance (%) and average size (standard length in centimeters), with standard error (SE) of each fish species captured in the eight studied lakes.

	%	Size (SE)	%	Size (SE)	%	Size (SE)	%	Size (SE)
Denmark	Lund Fjord (0.3‰)		Selbjerg (0.5‰)		Glombak (1.2‰)		Østerild (3.8‰)	
Roach, <i>R. rutilus</i>	84	5.5(0.1)	93	2.2(0.0)	50	2.8(0.2)		
Perch, <i>Perca fluviatilis</i>	16	4(0.2)	5	3.0(0.1)	45	3.9(0.1)		
European smelt, <i>Osmerus eperlanus</i>			1.3	2.9(0.1)	2.6	3.4(0.2)		
Three-spined stickleback, <i>G. aculeatus</i>			0.2	2.6(0)	1.2	2.2(0)	64	2.3(0.2)
Nine-spined stickleback, <i>P. pungitius</i>			0.5	2.1(0.2)			18	1.9(0.4)
Gobiidae							9.1	1.4(0.1)
Spain	Salins (0.4‰)		Sirvent (0.8‰)		Bassa Coll (1.6‰)		Ter Vell (2.2‰)	
Eastern mosquitofish, <i>G. holbrooki</i>	83	3.2(0.4)					74	2.5(0.0)
Sand smelt, <i>A. boyeri</i>			87	1.6(0.0)				
Thin-lipped grey mullet, <i>L. ramada</i>	0.3	5.2(0)	9.7	3.6(0.1)	67	2.9(0.1)		
Pumpkinseed sunfish, <i>Lepomis gibbosus</i>	15	3.4(0.3)	2.3	8.1(0.9)	24	4.5(0.3)	0.4	6.9(0.2)
European eel, <i>Anguilla anguilla</i>	1.4	33(7.0)	0.5	7.4(0)	7.0	16(4.8)	0.6	24(11.5)
Rudd, <i>Scardinius erythrophthalmus</i>	0.3	2.1(0)	0.5	7.4(0)	1.0	1.8(0)		
Flathead mullet, <i>Mugil cephalus</i>							25	4.7(0.1)
Goldfish, <i>Carassius auratus</i>					1.0	2.1(0)		

diate habitat, and daytime were selected as the baselines for the model. The selected variables included in the final model were obtained using an automatic stepwise selection, and the Akaike information criterion (AIC) was used to select the best model with increasing complexity. However, automatic stepwise procedures are rather generous about leaving terms in the model. Therefore, the increase in deviance caused by each variable included in the model and obtained with the stepwise selection was tested, and only variables causing a significant change in deviance were retained in the final model. An *F*-test was performed to obtain the significance of the variation explained by each selected variable because it is less sensitive to overdispersion problems (Crawley 2002). We excluded open-water sites in the GAM model since open-water data were lacking for two lakes, and for the remaining lakes the size distribution of intermediate and open-water sites showed high similarity, suggesting that zooplankton behaved similarly at both habitats.

Results

*Assemblage structure of potential zooplankton predators in Mediterranean and cold-temperate brackish lakes—Dif-*

ferent fish species were found in the set of lakes of Spain and Denmark, but in both regions the fish communities were characterized by few species and small-sized individuals (< 10 cm standard length), which potentially are important zooplankton predators (Mittelbach and Persson 1998; Blanco et al. 2003). The dominant fish species were eastern mosquito fish (*Gambusia holbrooki*), sand smelt (*Atherina boyeri*), and thin-lipped grey mullet (*Liza ramada*) in Spain, and in Denmark roach (*Rutilus rutilus*) and three-spined stickleback (*G. aculeatus*) (Table 2). Overall, mean fish densities were higher in Spain (Table 3) than in Denmark. However, apart from climate-related differences, there were some significant differences among lakes (significant effect of lake in the nested ANOVA, Table 3; Fig. 1). The highest mean fish densities were found in the Spanish lakes Ter Vell, Salins, and Sirvent together with the Danish lake Selbjerg, while the lowest fish densities were found in the Danish lakes Glombak and Østerild (Fig. 1).

Fish showed a positive association to submerged plants rather than to intermediate sites in both regions (significant effect of habitat in the nested ANOVA, Table 3), although the densities also depended on the lake and time of the day (Fig. 1). Fish occurred in higher densities within the plants

Table 3. Results of nested ANOVA on the effects of region (two levels, DK and SP), lake (four levels) nested inside region, habitat (two levels, S and I), and time (two levels, D and N) on the density of planktivorous fish and free-swimming macroinvertebrate (macroinv.) predators and of nested ANOVA on the effects of region, lake nested inside region, and time on the density of plant-associated macroinvertebrates. The region, habitat, and time with significantly higher densities are indicated in brackets: DK, Denmark; SP, Spain; I, intermediate areas; S, submerged plants; D, daytime; N, nighttime. Significance levels: \* *p* < 0.05, \*\*\* *p* < 0.0001.

	Fish		Free-swimming macroinv. predators		Plant-associated macroinv. predators	
	df	<i>F</i> values	df	<i>F</i> values	df	<i>F</i> values
Region	1	6.44* (SP)	1	3033.80*** (DK)	1	80.29*** (DK)
Lake (region)	6	5.73***	6	10.77***	6	16.36***
Habitat	1	62.66*** (S)	1	272.04*** (S)	—	—
Time	1	1.21	1	2.93	1	2.11
Error	118		118		55	

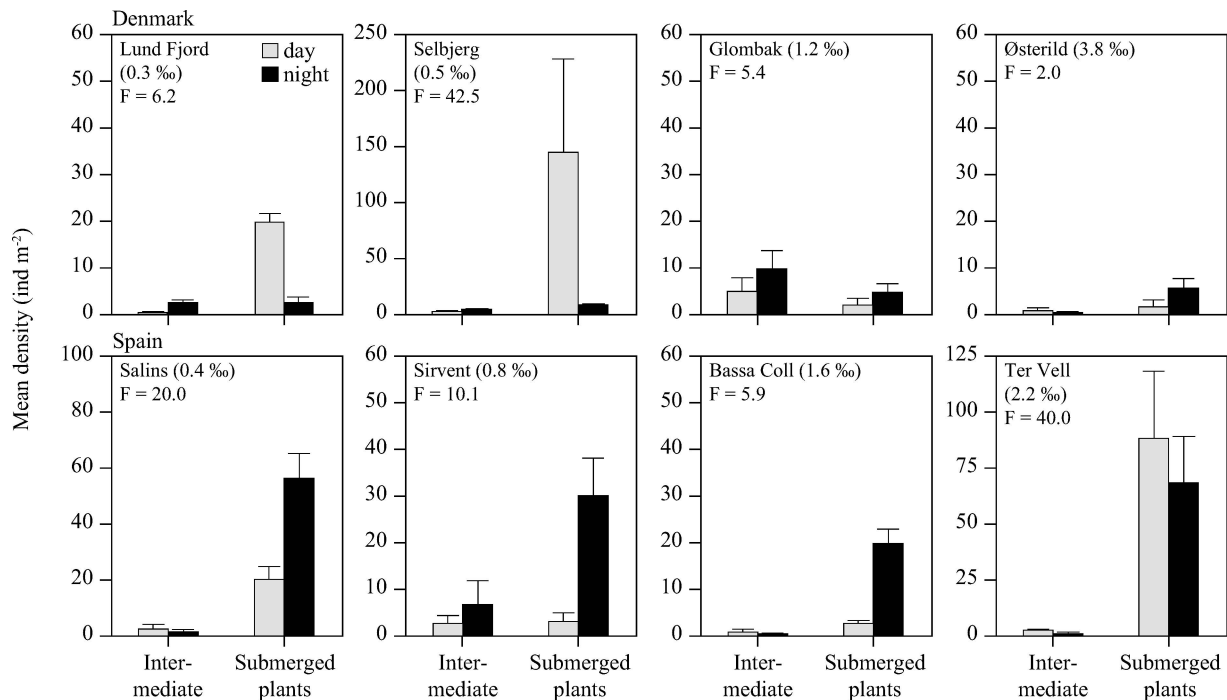


Fig. 1. Mean density (individuals  $m^{-2}$  [ $ind\ m^{-2}$ ]) and standard error (SE) of planktivorous fish in the intermediate and submerged plant habitats during day and night in each lake, with indication of the mean density for each lake (F). Note different scales for Selbjerg, Ter Vell, and Salins.

in all lakes except Glombak and Østerild, where similar densities among habitats were found (Fig. 1). In Lund Fjord and Selbjerg the differences between habitats disappeared at nighttime, while in Bassa Coll the differences disappeared at daytime.

Overall, macroinvertebrate predators showed a reverse pattern to that of fish since both the density of plant-associated macroinvertebrate predators and that of free-swimming macroinvertebrate predators were higher in Denmark than in Spain (Tables 3 and 4; Fig. 2). Climate-related differences were especially remarkable for free-swimming macroinvertebrate predators since they were scarce in most Spanish lakes. Only Bassa Coll, which had the lowest fish densities in Spain, had similar densities of plant-associated macroinvertebrate predators to Danish lakes (Fig. 2). Also a higher number of macroinvertebrate predator species occurred in Denmark than in Spain (Table 4). Like fish, free-swimming macroinvertebrate predators also occurred in higher abundances within the plants than at intermediate sites (significant effect of habitat in the nested ANOVA, Table 3). The pelagic invertebrate predator *Neomysis affinis* was highly abundant in the Danish lake Østerild and was also found, but in low densities, in Lund Fjord and Selbjerg (Table 4; Fig. 2). No differences of macroinvertebrate predator densities were found between daytime and nighttime.

**Zooplankton community structure**—Large-bodied cladocerans were almost absent in both regions. In Spain, the zooplankton communities of the four lakes were numerically dominated by rotifers, followed by nauplii at most of the sites (Fig. 3), whereas cladocerans and calanoid copepods

were almost absent. Cyclopoids were present in Sirvent and Bassa Coll, especially among plants, and were scarce in Salins. A zooplankton community mainly composed of Rotifera was found in the Ter Vell Lake, the Spanish lake with the highest fish density. Similar to the Spanish lakes, we found a zooplankton community dominated by rotifers and nauplii in Selbjerg, the Danish lake with the highest fish abundance (Figs. 1, 3). In contrast, small cladocerans were abundant in the three Danish lakes with lower fish abundances, Lund Fjord, Glombak, and Østerild.

Salinity was positively related to rotifer abundance and, in turn, to the abundance and biomass of total zooplankton, while it was negatively related to size diversity and the abundances of nauplii and cyclopoids (Table 5). Multiple regressions confirmed a negative relationship between planktivorous fish density and the total abundance and biomass of zooplankton, mean zooplankton body size, and zooplankton size diversity, at both intermediate and submerged plant sites (Table 5). Particularly, fish were negatively related to the abundances of cladocerans and cyclopoids at both habitats, and nauplii in the submerged plants, while they were positively related to rotifer abundance at the intermediate site. Free-swimming macroinvertebrate predators were negatively related to size diversity but positively related to the abundance and total biomass of zooplankton. Accordingly, a positive relationship was found between free-swimming macroinvertebrate predators and the abundances of rotifers, nauplii, and cladocerans at both habitats. Plant-associated macroinvertebrate predators were negatively related to the abundance and biomass of total zooplankton and the abundance of rotifers and cladocerans.

Table 4. Mean density (ind m<sup>-2</sup>), with standard error in brackets, of macroinvertebrate predators sampled by removing artificial plants (plant-associated macroinvertebrate predators) and sampled using the nets (free-swimming macroinvertebrate predators) in the eight studied lakes. Only species with an abundance of at least 1 ind m<sup>-2</sup> in one of the lakes are shown.

	Plant associated	Free swimming	Plant associated	Free swimming	Plant associated	Free swimming	Plant associated	Free swimming	Plant associated	Free swimming
	Lund Fjord (0.3%)		Selbjerg (0.5%)		Glombak (1.2%)		Østerild (3.8%)			
Denmark										
Hirudinea										
<i>Helobdella stagnalis</i>	350.9(33.0)	9.4(0.7)	4.0(0.0)	0.4(0.0)	4.0(1.4)	2.2(0.2)	0	2.2(0.2)	0	2.2(0.2)
<i>Eprobodella octocolata</i>	0	0	4.0(1.4)	0	0	0	0	0	0	0
<i>Glossiphonia heteroclita</i>	4.0(1.4)	0.1(0.0)	0	0.1(0.0)	0	0.1(0.0)	0	0	0	0
<i>Glossiphonia concolor</i>	4.0(1.4)	0.1(0.0)	0	0.2(0.0)	0	0(0.1)	0	0	0	0.1(0.0)
Arachnida										
Hydracarina	2227.5(135.3)	345.0(16.6)	290.4(25.4)	139.2(7.6)	641.4(36.7)	308.3(14.2)	44.0(1.4)	95.1(13.7)	0	0.7(0.1)
<i>Argyroneta aquatica</i>	0	1.8(0.2)	0	1.1(0.2)	0	1.0(0.2)	0	0	0	0
Brachitura										
<i>Argulus foliaceus</i>	0	0	0	10.2(1.2)	0	0	0	0	0	0
Malacostraca										
<i>Gammarus lacustris</i>	572.8(45.4)	162.9(12.3)	665.6(22.3)	282.8(18.1)	12.2(2.1)	4.1(0.3)	524.2(32.8)	942.8(63.4)	0	0
<i>Neonyxis integer</i>	0	3.7(0.3)	0	3.4(0.2)	0	0.5(0.0)	90	329.4(16.3)	0	0
Odonata										
<i>Coenagrion</i> sp. (nymph)	0	0.9(0.1)	56.5(5.2)	30.2(2.4)	76.6(4.8)	23.2(1.8)	4.0(1.4)	0.5(0.1)	0	0
Zygoptera (nymph)	0	0	4.0(1.4)	0	0	0	0	0	0	0
Heteroptera										
Corixidae (larvae)	0	15.7(0.9)	0	4.9(0.5)	0	6.1(0.5)	4.0(1.4)	2.0(0.2)	0	0
<i>Corixa</i> sp.	0	3.2(0.3)	0	0	4.0(1.4)	0.1(0.1)	0	55.2(3.9)	0	0
<i>Microvelia reticulata</i>	0	4.1(0.4)	0	1.0(0.2)	0	0.9(0.1)	8.1(1.9)	5.2(0.4)	0	0
Coleoptera										
Hydrophilidae (larvae)	0	0.5(0.1)	0	0.9(0.2)	0	1.5(0.1)	0	0	0	0
<i>Gyrinus</i> sp.	8.1(1.9)	0.1(0.0)	0	0	0	0	0	0.1(0.0)	0	0
Trichoptera										
<i>Cyrrus</i> sp. (larvae)	137.2(10.7)	1.5(0.2)	649.4(61.9)	25.9(6.2)	2238.7(90.8)	22.2(1.8)	4.3(1.4)	0.3(0.0)	0	0
Polycentropodidae (larvae)	0	35.8(2.6)	4.0(1.4)	227.6(20.0)	16.1(5.7)	517.1(35.9)	0	0.1(0.0)	0	0
<i>Oecetis</i> sp. (larvae)	8.1(2.9)	0.3(0.1)	0	0	0	0.1(0.0)	0	3.2(0.3)	0	0
<i>Trichostegia minor</i> (larvae)	0	0	0	0.3(0.0)	12.1(0.3)	0.2(0.0)	0	0	0	0
Phryganeidae (larvae)	4.0(1.4)	0	0	0.1(0.0)	0	0	0	0.1(0.0)	0	0
Diptera										
Tanypodinae (larvae)	88.(8.3)	28.3(1.4)	246.1(14.9)	91.7(4.3)	516.3(33.7)	197.7(11.2)	0	148.0(9.5)	0	0
Spain										
	Salins (0.4%)		Sirvent (0.8%)		Bassa Coll (1.6%)		Ter Vell (2.2%)			
Odonata										
<i>Ischnura elegans</i> (nymph)	13.1(14.6)	0	131.5(14.6)	0	0	0	0	0	0	0
Coenagrionidae (nymph)	0	0	0	2.1(0.4)	0	0.1(0.0)	0	0	0	0
Polychaeta										
<i>Nereis diversicolor</i>	0	0	0	0	13.1(4.4)	0	0	0	0	0
Malacostraca										
<i>Atyaephyra desmaresti</i>	0	2.1(0.2)	0	0	0	0	0	1.3(0.2)	0	0
<i>Leptocheirus pilosus</i>	0	0	0	0	2813.7(268.2)	0	0	0	0	0
Diptera										
Tanypodinae (larvae)	13.2(4.4)	0	39.4(6.5)	0	525.9(30.6)	0	144.6(18.8)	0	0	0



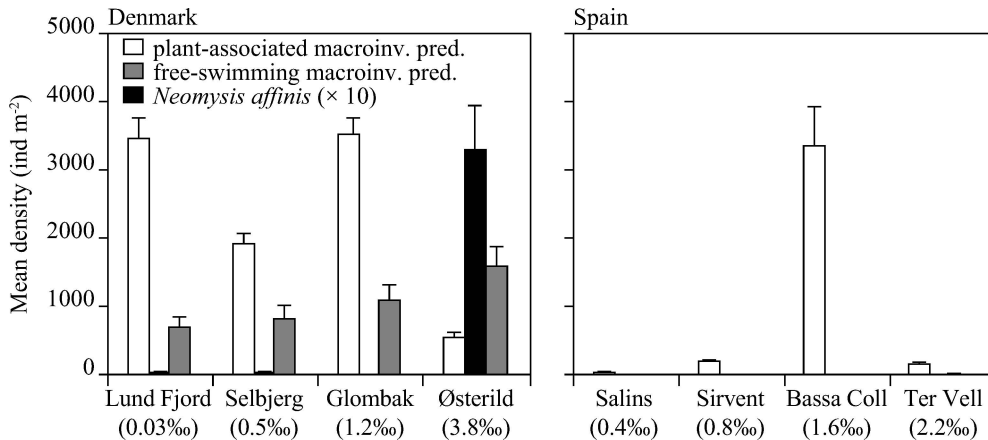


Fig. 2. Mean density (ind m<sup>-2</sup>) and SE of plant-associated macroinvertebrate predators and free-swimming macroinvertebrate predators for each lake, with a particular indication of densities of *N. affinis*.

The negative relationship between planktivorous fish density and zooplankton body size and abundance was reflected in the normalized biomass-size spectrum (Fig. 4) showing a unimodal shape with dominance of small sizes, mainly corresponding to rotifers, in the four lakes in Spain

(Figs. 4, 5). A similar shape was found in the Danish lake with the highest fish densities, Selbjerg. The rest of the lakes in Denmark showed a bimodal distribution, with a second dome (Kerr and Dickie 2001) corresponding to large sizes beginning around size class -2.5 (log<sub>2</sub> μg dry wt). The first

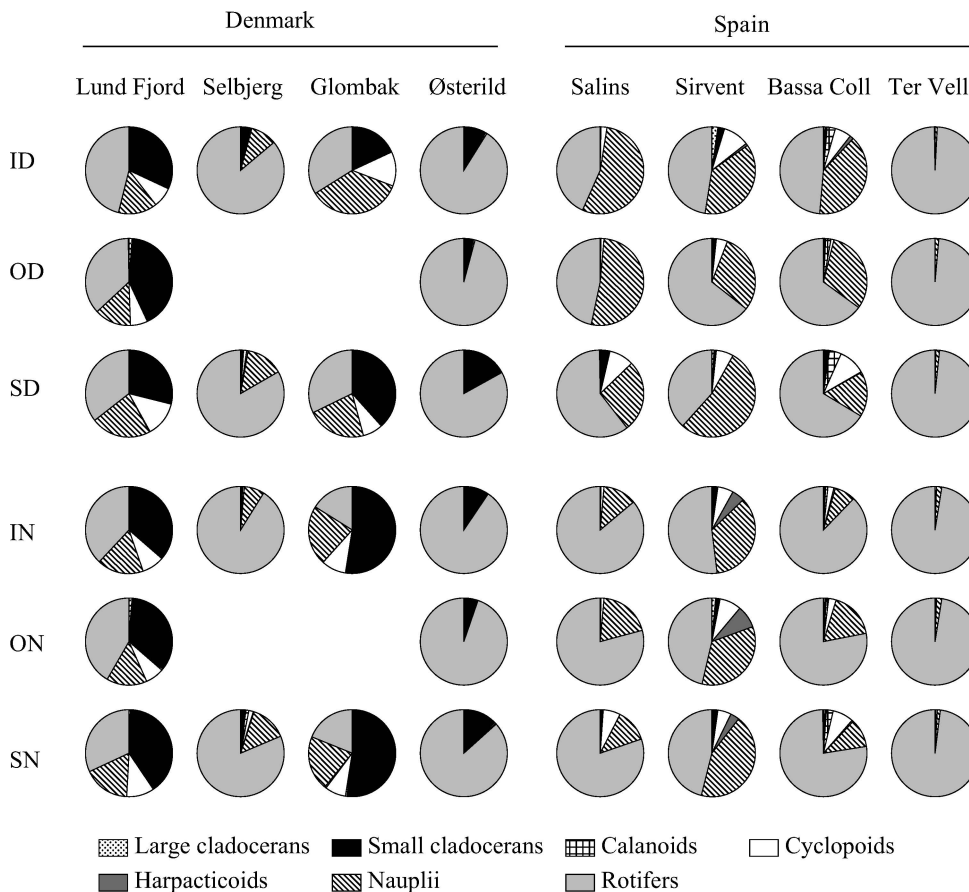


Fig. 3. Relative abundances of zooplankton groups in the different lakes and treatments. ID, intermediate site at daytime; OD, open-water site at daytime; SD, submerged plants site at daytime; IN, intermediate site at nighttime; ON, open-water site at nighttime; SN, submerged plants site at nighttime. Note: there were no open-water samples in Selbjerg and Glombak.



Table 5. Partial  $r^2$  from the stepwise multiple regression for zooplankton density and size distribution variables. Independent variables included were salinity, mean density of fish in the lake, mean density of fish in the habitat, mean density of free-swimming macroinvertebrate predators, and mean density of plant-associated macroinvertebrate predators. All variables were log-transformed. Significance level: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

Response variable	Salinity	Fish in lake	Fish in habitat	Free-swimming macroinv. predators	Plant-associated macroinv. predators
<b>Intermediate</b>					
Mean body size	—	-0.629***	—	—	—
Size diversity	-0.418**	—	-0.572***	-0.296*	—
Total abundance	0.429***	—	-0.421**	0.768***	—
Total biomass	0.452***	—	-0.381**	0.725***	—
Rotifer abundance	0.597***	0.571***	—	0.817***	—
Nauplii abundance	-0.565***	—	—	0.443***	—
Cyclopoid abundance	-0.481***	-0.410**	—	—	—
Cladoceran abundance	—	-0.619***	—	0.882***	—
<b>Submerged plants</b>					
Mean body size	—	—	-0.714***	—	—
Size diversity	-0.606***	—	-0.820***	-0.551***	—
Total abundance	0.691***	-0.540***	—	0.735***	-0.430***
Total biomass	0.486***	—	-0.700***	0.541***	-0.336**
Rotifer abundance	0.807***	—	—	0.813***	-0.612***
Nauplii abundance	-0.595***	—	-0.505***	0.450***	—
Cyclopoid abundance	-0.272***	—	-0.569***	—	—
Cladoceran abundance	—	-0.868***	—	0.884***	-0.453***

dome was mainly composed of rotifers and a few nauplii, while the second dome was mainly composed of small cladocerans and a few copepods (Fig. 5). The second dome corresponding to large sizes did not appear in the Spanish lakes and the Danish lake Selbjerg and, in accordance with their less evenly distributed size classes, they showed low size diversity (Fig. 5). A particular case was Østerild, where rotifers were highly abundant, thus creating a higher peak in the small sizes in the normalized size spectrum (Figs. 4, 5), but maintaining the bimodal distribution. The size range of zooplankton was similar in most of the lakes, the only differences being the relative abundances of the different sizes. The only exception was Østerild, where the largest

sizes (size class above  $0 \log_2 \mu\text{g dry wt}$ ) were nearly absent (Figs. 4, 5). Within each of the two regions, different salinities [e.g., Salins (0.4‰) and Ter Vell (2.2‰); Lund Fjord (0.3‰) and Glombak (1.2‰)] produced similar size distributions (Fig. 4).

The optimum GAM model for zooplankton normalized biomass with size class as a smoothing term explained 82.2% of the variance of the model (Table 6). GAM confirmed that lake was a significant source of variation for zooplankton normalized biomass, as were the interactions lake  $\times$  size class, lake  $\times$  habitat, and lake  $\times$  time. However, neither planktivorous fish nor macroinvertebrate predator densities were retained in the final model.

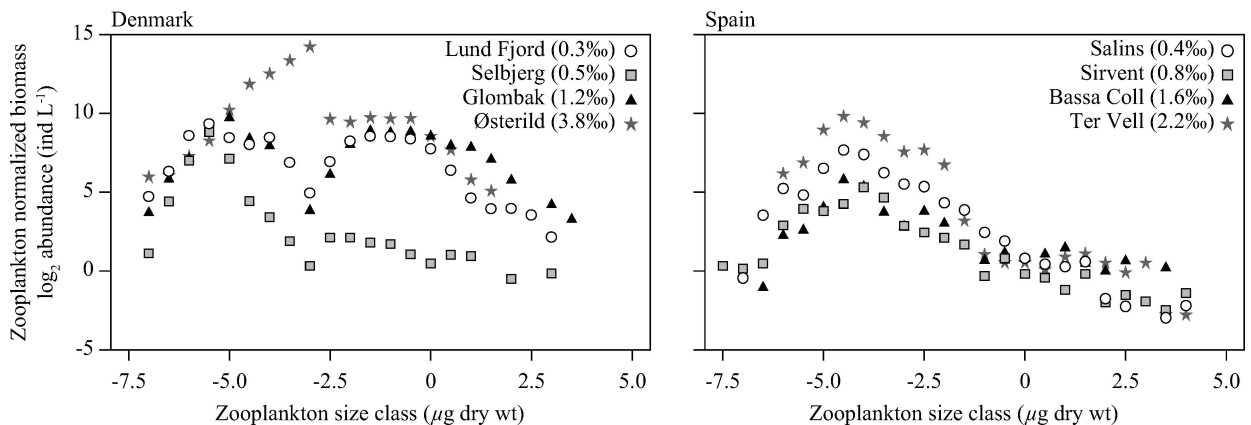


Fig. 4. Zooplankton normalized biomass-size spectra for each lake in Denmark and Spain. Values represent the average of the different replicates in each lake. Zooplankton size class represents  $\log_2$  (upper limit of size class) with size classes in micrograms dry weight. Normalized biomass represents  $\log_2$  (biomass in size class: width of size class), with biomass in micrograms dry weight, and is numerically close to the abundance of organisms per size class (individuals per liter).

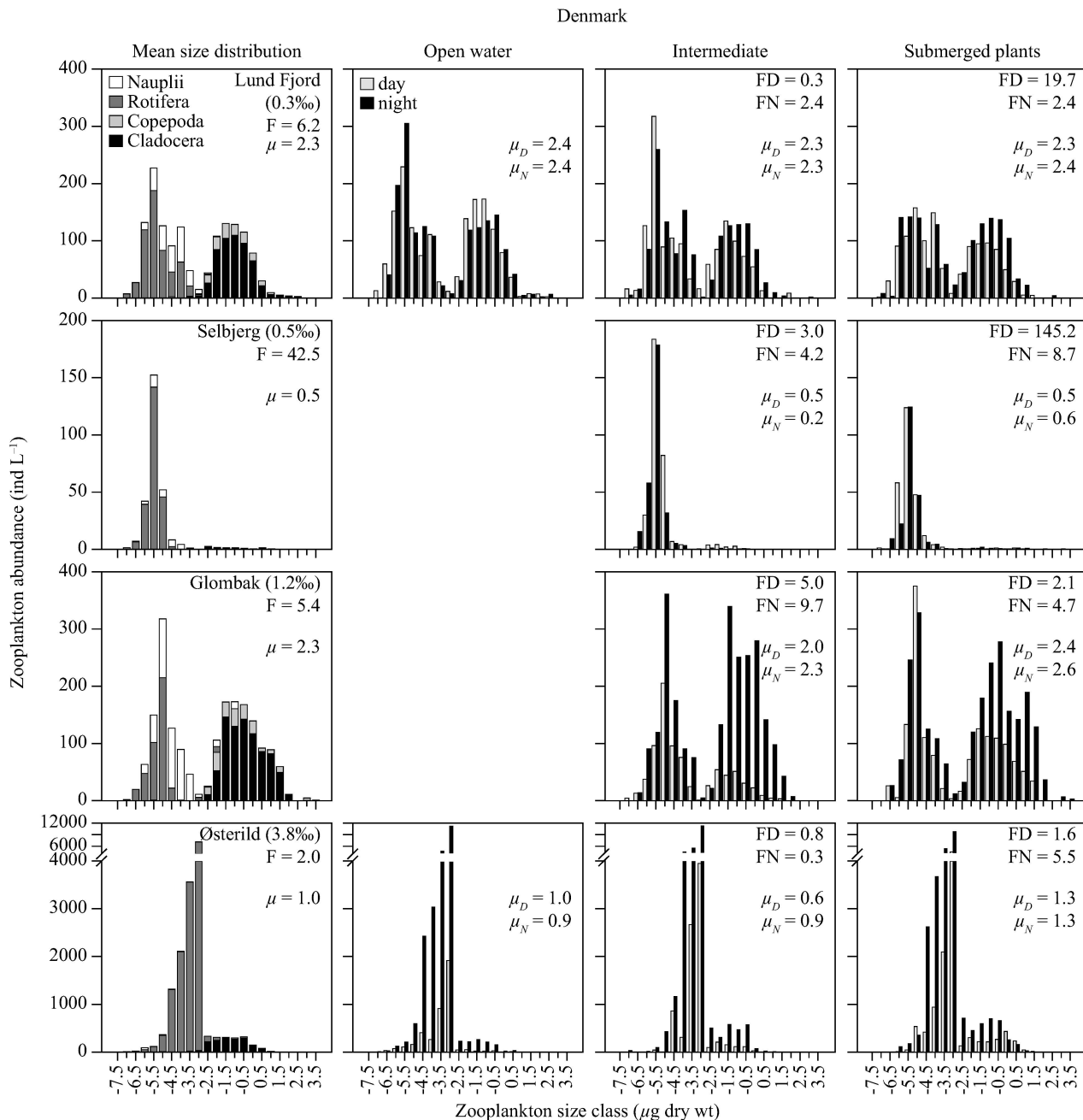


Fig. 5. Mean zooplankton size distribution for each lake (left column) with indication of abundances of nauplii, Rotifera, Copepoda and Cladocera, and zooplankton size distribution in the open-water, intermediate, and submerged plant sites during day and night for each lake (three columns on the right).  $\mu$ , mean zooplankton size diversity;  $\mu_D$ , zooplankton size diversity during day;  $\mu_N$ , zooplankton size diversity during night; F, mean fish density (ind m<sup>-2</sup>) in the lake; FD, mean fish density (ind m<sup>-2</sup>) during day; FN, mean fish density (ind m<sup>-2</sup>) during night. Note: there were no open-water samples in Selbjerg and Glombak.

Examination of the parametric coefficients for each lake, habitat, and time showed how size spectrum changed according to these factors (Table 7; Fig. 4). The intercept of the model for the Danish lakes Glombak, Lund Fjord, and Østerild, the lakes with lowest fish densities, was significantly higher ( $p < 0.001$  in all cases) than the baseline, Selbjerg (the lake with the highest fish density). In contrast, the intercept for the four Spanish lakes (Bassa Coll, Sirvent, Salins, and Ter Vell) was not significantly

different from the baseline. The interaction lake  $\times$  size class was significant, indicating that the relationship between size class and normalized biomass was not the same in the different lakes (Table 6). Thus, interaction terms confirmed that Selbjerg, Sirvent, Salins, and Ter Vell had similarly shaped normalized biomass-size spectra characterized by a unimodal distribution that peaked at small sizes (Fig. 4). They differed from the bimodal shaped normalized biomass-size spectra of Glombak, Lund Fjord, and

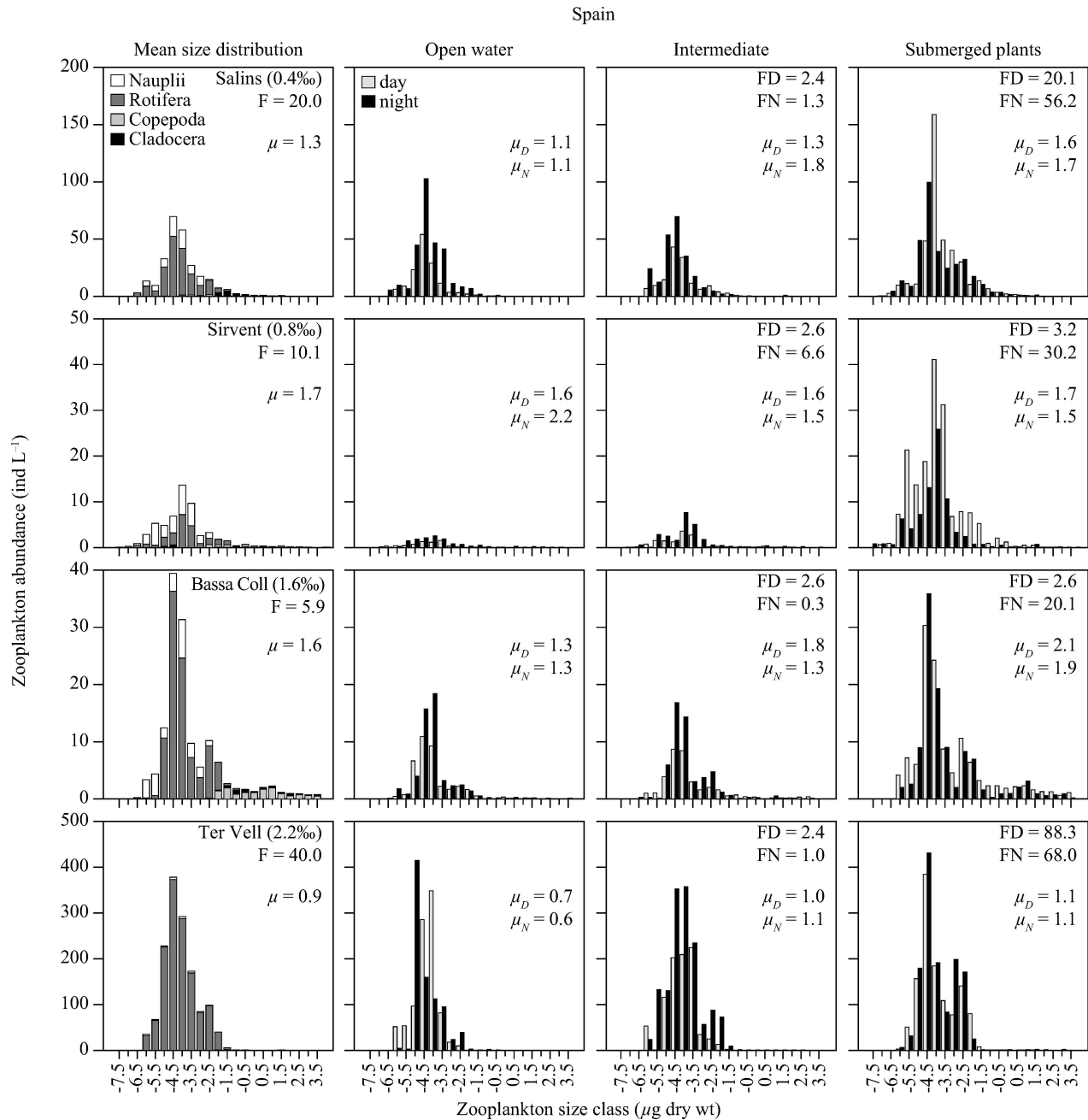


Fig. 5. Continued.

Østerild (interaction term lake × size class,  $p < 0.001$  for each lake) and the unimodal, but smoother, normalized biomass-size spectrum of the Bassa Coll (interaction term lake × size class,  $p < 0.01$ ).

The interaction lake × habitat was also a significant source of variation, particularly for Salins, Bassa Coll, and Sirvent in the submerged plants, which had significantly higher intercepts than other habitats (Table 7). The interaction lake × time also caused a significant variation in the model. The intercept was significantly higher in Glombak and Østerild during the night than during the day (Table 7). This is apparent in the size distributions of

Fig. 5, where higher abundances of nearly all size classes occurred during the night in both lakes.

### Discussion

Changes in the zooplankton composition and size distribution observed in coastal brackish lakes suggest a high dependence on the predation pressure by fish. Increased fish density coincided with a decrease of mean body size, size diversity, abundance, and biomass of zooplankton, most likely as a consequence of the size-selective predation toward large sizes. The result is

Table 6. Results for the optimum additive model. A normal (Gaussian) distribution was assumed for the response variable (zooplankton normalized biomass).

	df	<i>F</i>	<i>p</i>
Parametric terms			
Lake	7	62.52	<0.001
Habitat	1	0.08	0.776
Time	1	0.12	0.730
Lake × zooplankton size class	7	17.18	<0.001
Lake × habitat	7	4.62	<0.001
Lake × time	7	3.56	<0.001
Smoothing terms			
Zooplankton size class	6.18 (est.)	35.84	<0.001
Model parameters			
Deviance explained	82.2%		
Dispersion	2.95		
Residual degrees of freedom	538		
AIC	2295.8		

dominance of rotifers and some nauplii and a unimodal zooplankton size distribution that peaks at small sizes. Our results suggest that temperature per se does not explain the low abundances of large-sized zooplankton in the Mediterranean lakes, since low abundances were also found in the Danish Selbjerg lake with high fish densities. Hence, when fish predation was potentially high, the size structure of zooplankton was similar in both regions despite contrasting temperatures. Our results agree with previous studies showing that the fish community in temperate eutrophic brackish lakes is dominated by small macrophyte-associated fish species (Jeppesen et al. 1994) and give support to the hypothesis that in brackish eutrophic lakes macrophytes do not provide a proper daytime refuge to zooplankton against fish predation to the same extent as in temperate freshwater lakes dominated by larger planktivorous fish. The overall higher dominance of small planktivorous fish in the Mediterranean compared to the cold-temperate region likely reflects climate-related differences such as those observed in comparisons between freshwater lakes in temperate and subtropical regions (Meerhoff et al. 2007b; Teixeira-de Mello et al. 2009). Although the differences found between contrasting latitudes cannot be strictly ascribed to different temperatures, the patterns found might indicate the trends that temperate lakes may follow with climate warming (Meerhoff et al. 2007b).

We support the view that biomass-size spectrum is a useful tool for summarizing the effect of predation on zooplankton. Similar shaped biomass-size spectra, characterized by dominance of small sizes, were found in the lakes with high fish density regardless of the region (Selbjerg, Ter Vell, Salins, Sirvent), likely reflecting the intense fish predation on large specimens, which favors small-sized species. Accordingly, cladocerans and cyclopoids were negatively related to fish density both at intermediate and submerged plant sites, and rotifers were positively related to fish density at the intermediate sites. Our results also showed that higher intercepts of biomass-size spectrum

Table 7. Parametric coefficients calculated for the additive model for zooplankton normalized biomass. The model comprises a smoothing curve (zooplankton size class) and three nominal variables: lake ( $n = 8$ ), habitat ( $n = 2$ ), and time ( $n = 2$ ). Selbjerg, intermediate, and day are the baselines for the model.

	Estimate	Standard error	<i>t</i> value	<i>p</i>
Intercept	1.94	0.47	5.89	<0.001
Lake				
Sirvent	0.71	0.62	-3.90	0.052
Salins	2.04	0.65	-0.79	0.868
Bassa Coll	2.26	0.63	-2.09	0.605
Ter Vell	2.88	0.67	1.55	0.164
Glombak	7.64	0.66	5.43	<0.001
Lund Fjord	7.88	0.66	6.64	<0.001
Østerild	9.18	0.68	7.99	<0.001
Lake × habitat				
Sirvent × submerged plants	4.19	0.59	3.79	<0.001
Salins × submerged plants	3.60	0.61	2.70	<0.01
Bassa Coll × submerged plants	4.16	0.60	3.68	<0.001
Ter Vell × submerged plants	2.78	0.62	1.35	0.176
Glombak × submerged plants	2.57	0.61	1.03	0.305
Lund Fjord × submerged plants	2.10	0.61	0.26	0.794
Østerild × submerged plants	2.93	0.64	1.56	0.121
Lake × time				
Sirvent × night	1.55	0.58	-0.66	0.506
Salins × night	1.98	0.60	0.07	0.942
Bassa Coll × night	1.22	0.59	-1.21	0.225
Ter Vell × night	2.90	0.61	1.59	0.113
Glombak × night	3.14	0.60	2.15	<0.05
Lund Fjord × night	1.80	0.60	-0.23	0.816
Østerild × night	3.34	0.63	2.26	<0.05

were related to lower fish densities and vice versa. In the lakes with the highest fish densities the size distribution of zooplankton did not vary between day and night or between habitats with and without plants, probably as a result of the high fish predation, which tends to homogenize the biomass-size spectrum. This holds true even when the association of fish with macrophytes changed over time, as was the case in Selbjerg where fish aggregated within the macrophytes only during day. The remarkably higher abundances of zooplankton during night than during day in the Danish lakes Glombak and Østerild could reflect that zooplankton avoid the pelagic habitats during the day when the risk of predation is higher. These diel patterns were only observed in the lakes with fewer fish, lending further support to the hypothesis that increased predation pressure leads to a more homogeneous size distribution. The contrasting zooplankton size structure in lakes with different predation pressure and the shifts in zooplankton size structure between day and night differ substantially from the similar and stable zooplankton size structure reported by Sprules (2008) for a temperate North American and a tropical African freshwater lake. However, in this case, lakes were deep and had similar fish densities, which could be the reason for their similar zooplankton size structure.

Salinity apparently had a partial effect on the structure of the zooplankton community. It was negatively related to



size diversity as a consequence of the increase in rotifer density and decrease of cyclopoids at higher salinities. However, it was not related to zooplankton mean body size, and similar size spectra were found in lakes with contrasting salinities, probably reflecting the narrow salinity range of the lakes of our study. In a previous study in the same regions covering a larger salinity range (from 0.3‰ to 55‰), a shift was observed from dominance of large cladoceran species to dominance of copepods and small cladoceran species with increasing salinities (Bruce et al. 2009). In the present study, the zooplankton was dominated by small Cladocera, copepods, and rotifers, which are typical of eutrophic brackish systems (Jeppesen et al. 1994; Bruce et al. 2009). Large Cladocera, such as *Daphnia* and *Simocephalus* spp., were also present, though in very low abundances. Both genera have been reported at salinities up to 8‰ in temperate and in Mediterranean brackish ecosystems (Bruce et al. 2009). Thus, the majority of the cladoceran species present could tolerate salinities higher than those recorded in the study lakes. Nevertheless, salinity seems to play a more indirect role in the trophic structure of brackish lakes by determining fish communities and, in turn, shaping zooplankton community structure. Dominant fish species such as three-spined stickleback, nine-spined stickleback, eastern mosquito fish, and sand smelt are tolerant to the salinity ranges of this study and common in brackish waters (Jeppesen et al. 1994), while the freshwater species roach, which was highly abundant in the Danish lake Selbjerg, is very common in northern brackish coastal areas (Härmä et al. 2008). Several studies have shown that these species prey intensively on zooplankton (García-Berthou 1999, 2000; Blanco et al. 2003). According to experimental studies (Jakobsen et al. 2003), fish densities above 4 to 6 stickleback  $m^{-2}$  can lead to substantial changes in the zooplankton community structure and may cause a shift from a clear to a turbid state in temperate brackish lakes. In our study, higher fish densities were found in Selbjerg, Ter Vell, Salins, and Sirvent Lakes.

The commonly reported higher densities of piscivorous fish in cold-temperate regions compared to Mediterranean regions (Blanco et al. 2003) could explain the observed different diel patterns of planktivorous fish in Danish and Spanish lakes. Thus, in Denmark, the relatively high daytime planktivorous fish densities in the submerged plants could be ascribed to the presence of the piscivorous perch (*Perca fluviatilis*) and pike-perch (*Stizostedion lucioperca*) in the open water as reported in previous studies in these lakes (Jeppesen et al. 2002). In the Spanish lakes, eel (*Anguilla anguilla*) is the only potentially piscivorous fish common in all four lakes (Moreno-Amich et al. 1996), although fish represents only less than 10% of its diet (Costa et al. 1992; Blanco et al. 2003), while European seabass (*Dicentrarchus labrax*) has been occasionally reported in Sirvent Lake.

Climate-related differences in fish communities might have influenced the abundance of macroinvertebrate predators, which tended to be more abundant in the cold-temperate region than in the Mediterranean region. Only Bassa Coll, the Spanish lake with fewer fish, held a similar density of plant-associated macroinvertebrate predators to

that of the Danish lakes, mainly due to the presence of the omnivorous *Leptocheirus pilosus* (DeWitt et al. 1992). A pattern of higher abundance of fish co-occurring with lower abundance of macroinvertebrates in subtropical freshwater lakes compared to similar temperate lakes was also observed by Meerhoff et al. (2007b) in a similar cross-latitudinal experiment. In our study, this pattern would explain the apparent positive relationship between the abundance of free-swimming macroinvertebrate predators and all the zooplankton groups except cyclopoids. The remarkably higher abundances of rotifers in the Danish lake Østerild compared to the other study lakes could be explained by the high densities of *N. integer* preying on the largest size classes, including cyclopoids, and thereby releasing predation on rotifers. In contrast to free-swimming macroinvertebrate predators, plant-associated macroinvertebrates apparently had a negative effect on the abundance of rotifers and cladocerans and, in consequence, were negatively related to the total abundance and biomass of zooplankton. However, they did not affect zooplankton mean size.

Despite the fact that the size spectra approaches are cost efficient and easy to use, researchers may find it difficult to determine significant differences among size distributions. This is particularly difficult for nonlinear distributions, which are a common feature of the size spectrum of the functional groups (e.g., phytoplankton, zooplankton, and fish; namely, the *ecological scaling* of the size spectrum, Kerr and Dickie 2001; Bruce et al. 2005a,b). In consequence, most ecological studies have used size spectrum merely as a descriptive tool. In that sense, GAM analysis provides a useful means for testing differences between size spectra (De Eyto and Irvine 2007). In our study we included environmental factors (e.g., fish density) in the GAM analysis in order to test their effect on normalized biomass; however, these were not retained in the final model. If so, size diversity provides a unique value for size distribution that can be used to identify the relative importance of environmental variables. Here a decrease in zooplankton size diversity with increasing fish predation indicated that strong fish predation concentrates zooplanktoners in a very narrow size range. On the other hand, with decreasing fish predation, zooplankton size distribution becomes smoother and larger in range, that is, more diverse. Similar effects of fish predation on zooplankton size diversity have previously been described by Badosa et al. (2007).

*Implications under a climate-change scenario*—Recent studies in freshwater shallow lakes have shown that one of the effects of climate warming may arise through the enhanced fish predation pressure triggered by higher temperatures (due to smaller size and higher density of fish resulting from enhanced reproduction and higher activity level), which would negatively affect the resilience of the lakes to enhanced nutrient loading by decreasing the density of large-bodied zooplankton and thus the top-down control on phytoplankton (Moss et al. 2004; Romo et al. 2004; Meerhoff et al. 2007b). Our results agree, since the structure of the predator assemblages, with higher dominance of small fish in the Mediterranean region, was the

main reason for the observed differences in zooplankton community structures in the lakes under study. As reported for warm (subtropical) freshwater lakes (Meerhoff et al. 2007a,b), in brackish lakes, fish and macroinvertebrate predators are highly associated with the submerged plants; thus we cannot expect the predation pressure on zooplankton to diminish with increasing habitat complexity. Furthermore, our study, together with a previous cross-comparison study of brackish lakes in the same regions (Bruçet et al. 2009), showed that salinity may influence zooplankton size structure toward predominance of small-sized zooplankton and a decrease in species richness. From a global-warming perspective, these results suggest that changes in the trophic structure of brackish lakes in temperate regions might be expected as a result of the warmer temperatures and the potential associated increases in salinity. It is likely that the effect of salinity on the trophic structure explains the relatively more homogeneous zooplankton community observed in brackish lakes along a latitudinal gradient compared to that of freshwater lakes.

We have provided experimental evidence that both size diversity and the biomass-size spectrum at a functional scale shift relative to predation pressure and salinity, regardless of climate and taxonomic composition. Since body size is related to variables describing the main ecological processes (e.g., respiration, production, growth rate, ingestion rate) (Kerr and Dickie 2001), size approaches appear as promising tools to assess ecological change induced by climate-change effects and/or human disturbances (e.g., temperature and salinity shifts, loss of top predators, switches between alternative states) and ought to receive more attention in future studies.

#### Acknowledgments

We thank J. Sala, J. Compte, D. Balayla, P. Hospital, J. Puig, A. Ruhi, N. S. Laursen, D. A. Christiansen, L. Thuesen, T. Skov, F. K. Yousfi, N. Christiansen, B. Birkeland, and C. Katborg for assistance in the field; the staff of the National Environmental Research Institute and C. Conchillo for technical and analytical assistance; T. Christensen, J. Jacobsen, and A. M. Poulsen for editorial assistance; E. de Eyto for statistical assistance; and R. W. Sterner and two anonymous reviewers for valuable comments that helped shape this paper.

Further support was obtained in Denmark from the projects "Eurolimpacs" (EU), "WISER" (EU), The Research Council for Nature and Universe (272-08-0406), and the Centre for Regional Change in the Earth System, and in Spain from Ministerio de Educación y Ciencia, Programa de Investigación Fundamental (GL2008-05778/BOS). S.B. held a postdoctoral grant from the Dept. d'Universitats, Recerca i Societat de la Informació de la Generalitat de Catalunya. M.M. was supported by the Uruguayan Programme for the Development of Basic Sciences and the National System of Researchers (Uruguayan Agency for Research and Innovation, Uruguay).

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Associate editor: Robert W. Sterner

Received: 15 September 2009

Accepted: 01 March 2010

Amended: 16 April 2010