

RESEARCH ARTICLE

Taxonomic and dimensional structure of benthic macroinvertebrate guilds in the Margherita di Savoia Salt Pans (Italy)

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Abstract

- 1 - The importance to community assembly of species selection mechanisms based on functional traits (i.e. niche filtering) is the object of intense debate. In this study, the influence of selection mechanisms was estimated in an ecosystem, the Margherita di Savoia Salt Pans, where the salinity gradient is expected to constitute a strong selective constraint.
- 2 - The spatial and seasonal variations of organisation of the macrobenthic communities was studied along a salinity gradient. At the taxonomic level, species richness, numerical abundance of individuals and diversity were found to vary with gradient levels while not directly with salinity.
- 3 - Under the most extreme conditions (the highest gradient level) benthic macroinvertebrate guilds became highly simplified, with *Chironomus salinarius* being dominant.
- 4 - In terms of dimensional structure, the individuals sampled ranged across five orders of magnitude, and were divided into 19 octaves of abundance. In general, size-abundance distributions indicated equal resource partitioning with body size; the distributional parameters showed significant variations with salinity.
- 5 - The relative importance of the main “niche oriented” and “body size oriented” organization mechanisms of benthic macroinvertebrates guilds along the salinity gradient was evaluated and discussed.

Keywords: benthic macroinvertebrates, hypersaline ecosystems, niche oriented models, body size oriented models, community organization.

Introduction

The study of community organization mechanisms is one of the main topics in ecology. Approaches to the study of these mechanisms may be based on different premises. We briefly describe the “niche oriented” and the “body size oriented” approaches here.

The “niche oriented” approach has its conceptual origins in niche theory (Hutchinson, 1957) and is based on resource partitioning as a premise for species coexistence. Niche-apportionment models (Tokeshi, 1999) are an example of the application of this approach which allow for speculation about the factors

which regulate competitive coexistence. These models are based on the direct proportionality between species abundance and the niche space occupied by the species, assuming equivalence of species metabolic rates.

In contrast, the “body size oriented” approach attributes a determinant role to body size in controlling species coexistence dynamics (Hutchinson, 1959; Basset, 1995). An example of this approach is the study of size-abundance relationships, which is based on the “energetic equivalence rule” (EER) (Damuth, 1981).

In this work, both “niche oriented” and “body size oriented” approaches are used and compared in order to describe organization

mechanisms in an ecosystem, the Margherita di Savoia Salt Pans, characterized by a strong salinity gradient.

Specifically, the aim of this study is 1) to describe variation patterns in the structure of benthic macroinvertebrate guilds along a salinity gradient and 2) to evaluate the presence, along the same gradient, of variation of community organization mechanisms in the Margherita di Savoia Salt Pans.

Materials and Methods

The Margherita di Savoia Salt Pans are located along the south-eastern coast of the Adriatic Sea (41.24°N; 16.04°E). The total surface area is about 40Km² with a mean depth of 2.5m. They are connected to the Adriatic Sea by a channel 2350m long and about 4m deep. The Salt Pans consists of two different basins linked to each other: the first has a surface area of 35Km² and is used for the process of evaporation, while the second, with a surface area of 5Km², is used for the process of salinization. The bottom of the Margherita di Savoia salt pans where sampling was conducted is muddy without vegetation.

Sampling

Changes in the macroinvertebrate community along the salinity gradient were investigated using a nested experimental design, in which the seasons and the salinity gradient were factors of variability (independent) and the stations were nested in each level of the gradient. The

experimental design consisted of two seasonal samplings, four salinity gradient levels, two stations for each gradient level and five replicates for each station. Benthic macroinvertebrates were collected in fall 2004 and spring 2005, corresponding to two different stages of the salt pan production cycle. In spring, the channel connecting the salt pans to the sea is opened and sea water is pumped in; the channel is closed at the beginning of summer. The fall corresponds to the final period of evaporation and maximum salinity.

In the present study, the choice of gradient levels and the positioning of the stations at each level of the gradient were based on the fall salinity values (Tab. 1). The stations were situated 76m to 2720m away from the marine water input (Tab. 1 and Fig. 1). The samples were collected using a box-corer (0.03m²), each sample was sieved through a 0.5mm mesh sieve and stored in 4% formalin solution. In the laboratory, the collected benthic macrofauna was sorted and selected under a stereomicroscope. Animals were later identified to species level where possible. Each individual was weighed to the nearest $\pm 1\mu\text{g}$ after drying for 72h at 60°C. For the determination of ash, groups of co-specific individuals were incinerated in a muffle furnace for 4h at 500°C. Biomass estimates were expressed as ash free dry weight (AFDW).

Table 1. List of stations showing salinity values, gradient levels and distance from sea water input (A).

Gradient	Salinity (psu)	Stations	Distance (m)
Level 1	56.40	1	1260
		2	1218
Level 2	58.21	3	76
		4	87
Level 3	64.22	5	747
		6	760
Level 4	86.78	7	2720
		8	2530

Data analysis

Macrobenthic community structure was analyzed in terms of density of individuals (N/m^2), species richness according to the Margalef index (Margalef, 1958), species diversity according to the Shannon index (Shannon and Weaver, 1949) and Taxonomic Distinctness (Warwick and Clarke, 1995), the indices being calculated for each sampling unit (replicate) using the Primer software package. Nested Anova analyses of variance were used to evaluate the spatial variation of numerical density (N/m^2) and the above-mentioned indices. The model takes account of two factors: "gradient" was treated as fixed factor, while "station" was considered as a random factor nested within "gradient". The two seasons were analysed separately. In order to estimate the differences between the levels of factors, the Tukey test (STATISTICA package) was applied. Data on individual masses were logarithmically transformed (\log_2) and grouped into size classes in order to describe the body size structure of benthic macroinvertebrate guilds. The statistical parameters of the size

distributions analyzed in this study were width (calculated as the difference between the 75th and the 25th percentile) and median.

The study of body-size oriented mechanisms of community organization looked at the size-abundance relationships via regression analysis between population density and average size of each species on a bi-logarithmic scale. The slope of the regressions (b) was compared (parallelism test) to the expected value of the energetic equivalence rule of $b=-0.75$.

The study of the niche oriented mechanisms of community organization compared species abundance distributions of sampled benthic macroinvertebrates with theoretical distributions expected from five niche apportionment models proposed by Tokeshi (1999). The five models considered were Dominance Decay (DD), Dominance Pre-emption (DP), MacArthur Fraction (MF), Random Assortment (RA) and Random Fraction (RF). The distribution fitting process was carried out according to the method proposed by Mouillot et al (2003).

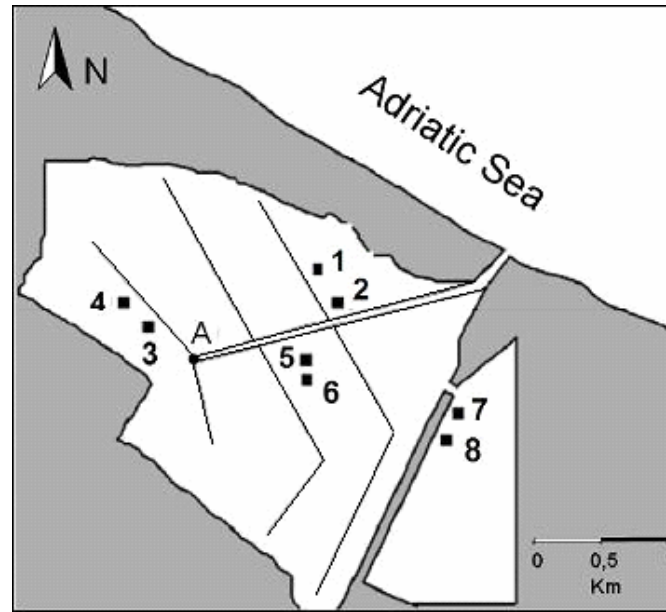


Figure 1. Study area with location of sampling stations and sea water input.

Results

Structural features of benthic macroinvertebrate guilds.

In total, 9727 individuals were collected, of which 5273 individuals (54.17%) in spring and 4464 (45.83%) in fall, belonging to 39 taxa, 15 of which were at species level. 95% of macroinvertebrates belonged to 7 taxa (Tab. 2). Of these, 48.39% were *Chironomus salinarius*. The most abundant species were also the species present in the greatest number of sampling units. The abundance and the frequency of the species (the latter measured as the percentage of stations where the species were found) were positively related ($y=23.7*x^{2.77}$, $R^2=0.69$,

d.f.=37, $P<0.01$; y =frequency, x =abundance). The abundance of species also was, on a bi-logarithmic scale, inversely related to average organism size ($y=9.79*x^{-0.41}$, $R^2=0.11$, d.f.=35, $P<0.05$; y =species abundance, x =average organism size). Overall, the individuals' average size varied between seasons, being larger in spring than in fall (Student's t-test, $t=1.96$, d.f.=9733, $P<0.05$) (Fig. 2). The slope (b) of the relation between average size and numerical abundance was significantly different from the value $b=-0.75$ expected in accordance with the energetic equivalence hypothesis ($P<0.05$).

Table 2. Abundance, relative frequency and mean size of 39 taxa found in the study area.

Taxa	Abundance %	Frequency (%)	Average size (mg)
<i>Chironomus salinarius</i>	48.39	87.50	0.65
<i>Hydrobia ventrosa</i>	17.20	31.25	0.24
<i>Corophium</i> sp.	10.99	56.25	0.06
<i>Gammarus insensibilis</i>	9.24	75.00	0.66
<i>Microdeutopus gryllotalpa</i>	3.88	31.25	0.06
Oligochaeta	2.84	25.00	0.18
<i>Loripes lacteus</i>	1.92	25.00	5.20
<i>Malacoceros fuliginosus</i>	0.67	6.25	0.34
<i>Capitella capitata</i>	0.58	18.75	6.17
<i>Cerastoderma glaucum</i>	0.58	25.00	5.88
<i>Microdeutopus</i> sp.	0.45	12.50	0.06
<i>Abra segmentum</i>	0.43	37.50	16.39
Lumbrinereidae	0.42	12.50	5.16
<i>Naineris laevigata</i>	0.34	12.50	8.24
Polychaeta	0.32	18.75	6.32
Actiniaria	0.26	12.50	1.19
Chironomidae pupa	0.26	12.50	0.93
Nereididae	0.25	31.25	3.36
Spionidae	0.24	18.75	1.26
<i>Nereis diversicolor</i>	0.20	50.00	7.37
<i>Tellina</i> sp.	0.16	12.50	18.32
<i>Artemia salina</i>	0.06	18.75	0.29
<i>Bittium reticulatum</i>	0.03	6.25	11.98
Gammaridae	0.03	18.75	0.55
<i>Haminoea</i> sp.	0.03	6.25	16.63
<i>Sphaeroma hookeri</i>	0.03	12.50	3.75
<i>Tapes</i> sp.	0.03	12.50	94.79
Cypraeidae	0.02	6.25	3.95
Lepidoptera	0.02	12.50	7.56
Lumbricidae	0.02	6.25	16.78
<i>Nassarius</i> sp.	0.02	6.25	25.12
Nematoda	0.02	6.25	0.06
Ceratopogonidae	0.01	6.25	0.22
Cirratulidae	0.01	6.25	3.55
<i>Idotea baltica</i>	0.01	6.25	0.29
Insecta	0.01	6.25	/
Planariidae	0.01	6.25	/
<i>Sphaeroma monodi</i>	0.01	6.25	5.01
Stratiomyidae	0.01	6.25	0.90

Analysis of spatial variation

In fall, the numerical density and the taxonomic descriptors (species richness and species diversity, the latter including taxonomic distinctness) of benthic macroinvertebrate guilds varied significantly among the gradient levels. In spring the variation was limited to species richness and species diversity (nested ANOVA, Tab. 3). The variation of these

descriptive features was directional, being influenced by both the salinity gradient and the distance from marine water input.

Indeed, all the features taken into account decreased with salinity and distance in both spring and fall except for the density, which in fall was directly proportional to salinity ($P < 0.05$).

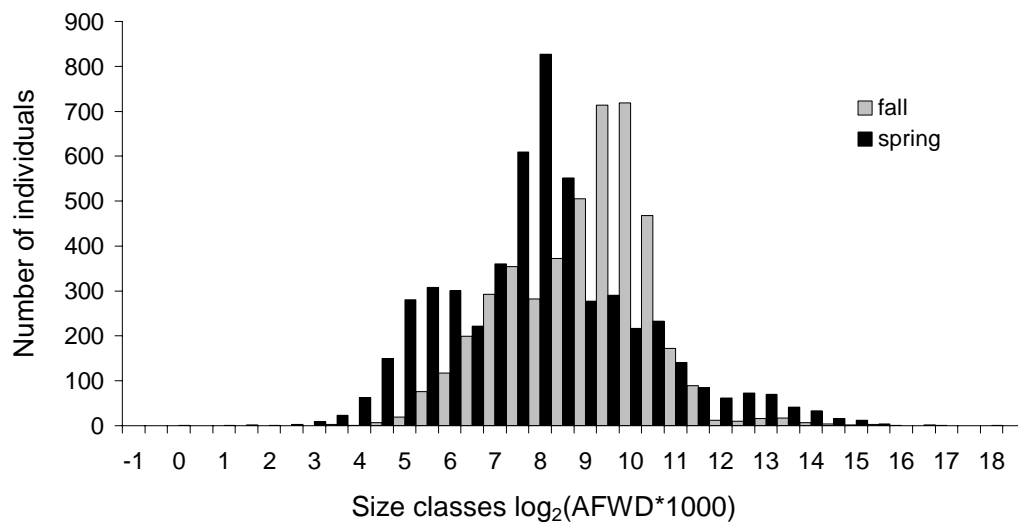


Figure 2. Size distribution of benthic macroinvertebrates in two sampling seasons.

Regarding the dimensional component, the width of size distributions (measured as the range between the 25th and the 75th percentile) decreased with salinity ($P < 0.05$) in spring but not in fall. For both seasons there was a decrease in the width of size distributions with increasing distance.

Median size distribution increased with salinity and distance in both seasons, the relationship between distance and the median being significant only in the fall ($P < 0.05$).

Analysis of guild organization

In order to test the body-size oriented approach, the slopes (b) of the size-abundance relationships were compared with the expected value of -0.75, in accordance with the energetic

equivalence hypothesis. The slopes are shown in Table 4; in this table the values relative to the fourth salinity level in fall and in one of the two spring stations are not shown because species richness is too low for regression analysis. In spring, the values of the slopes were higher than -0.75 in the stations of the first two gradient levels and lower in station 7 of the fourth level (Tab. 4). In the stations of the third level, the slopes did not differ significantly from -0.75. In fall, the slopes were higher than -0.75 in the second level stations but not significantly different in the third and in the fourth level stations. Negative relationships were found between the slopes and salinity (and between the slopes and distance from the entry point of sea water) only in spring ($P < 0.05$).

Table 3. Density (N/m²) of each taxa at each gradient level in both sampling seasons and values of community descriptor indices (richness, diversity, taxonomic diversity) at each level in both seasons. The P values indicate significant differences among gradient levels (**P<0.01; ***P<0.001; n.s.=not significant).

Taxon	Spring				P	Fall				P
	Gradient levels					Gradient levels				
	1	2	3	4		1	2	3	4	
<i>Abra segmentum</i>	6.92	124.57	3.46		n.s.			10.38		***
Actiniaria		86.51			**					***
<i>Artemia salina</i>		3.46			***		17.30			***
<i>Bittium reticulatum</i>			10.38		n.s.					***
<i>Capitella capitata</i>					n.s.		128.03	65.74		***
<i>Cerastoderma glaucum</i>	152.25		27.68		n.s.			13.84		***
Ceratopogonidae				3.46	n.s.					***
Chironomidae pupa				86.51	n.s.					***
<i>Chironomus salinarius</i>	86.51		584.78	1525.95	n.s.	889.27	17.30	3453.29	9747.40	***
Cirratulidae					n.s.		3.46			***
<i>Corophium</i> sp.	103.81	2948.10	6.92		n.s.	83.04	560.55			***
Gammaridae	3.46	3.46	3.46		n.s.					***
Cypraeidae					n.s.		6.92			***
<i>Gammarus insensibilis</i>	138.41	429.07	2128.03	332.18	n.s.	13.84	6.92	65.74		***
<i>Haminoea</i> sp.		10.38			n.s.					***
<i>Hydrobia ventrosa</i>	2605.54	55.36	3134.95		n.s.					***
<i>Idotea baltica</i>					n.s.		3.46			***
Insecta				3.46	n.s.					***
Lepidoptera			3.46	3.46	n.s.					***
<i>Loripes lacteus</i>		605.54			n.s.		34.60	6.92		***
Lumbricidae					n.s.		6.92			***
Lumbrinereidae		141.87			n.s.					***
<i>Malacoceros fuliginosus</i>					n.s.		224.91			***
<i>Microdeutopus gryllotalpa</i>	629.76	660.90			n.s.		17.30			***
<i>Microdeutopus</i> sp.	152.25				n.s.					***
<i>Nainereis laevigata</i>		114.19			n.s.					***
<i>Nassarius</i> sp.			6.92		n.s.					***
Nematoda		6.92			n.s.					***
Nereididae	65.74	6.92	10.38		n.s.					***
<i>Nereis diversicolor</i>	3.46	3.46	6.92	3.46	n.s.	3.46	44.98			***
Oligochaeta	470.59		487.89		n.s.					***
Planariidae	3.46				n.s.					***
Polychaeta	3.46	103.81			n.s.					***
<i>Sphaeroma hookeri</i>					n.s.		10.38			***
<i>Sphaeroma monodi</i>		3.46			n.s.					***
Spionidae	69.20				n.s.			10.38		***
Stratiomyidae				3.46	n.s.					***
<i>Tapes</i> sp.		10.38			n.s.					***
<i>Tellina</i> sp.		27.68	27.68		n.s.					***
Density (N/m ²)	4494.81	5346.02	6442.91	1961.94	n.s.	989.62	1083.04	3626.30	9747.40	***
Margalef index	0.87	0.78	0.54	0.31	**	0.15	0.49	0.16	0	***
Shannon index	1.14	1.22	1.10	0.65	***	0.30	0.91	0.19	0	***
Taxonomic Distinctness	85.79	79.46	97.76	69.43	n.s.	71.11	197.29	94.44	0	***

In spring, among the five niche apportionment models, applied to each gradient level, the Random Assortment model could not be rejected for the first three gradient levels (Tab. 5), the second gradient level showing the best fit (the largest P value). At the fourth level, the Dominance Pre-emption model was the only model accepted. A graphic representation of the

fit of these two models at all four gradient levels is shown in Figure 3. In fall, models could be accepted only at the second gradient level (Random Fraction and Random Assortment). For the first and third level, all proposed models were rejected. At the fourth level, where only one species was found, any model could not be applied.

Table 4. *b* values of size-abundance relationships for each station. Asterisks indicate slopes that are different from the expected value of -0.75 (**P*<0.05).

Gradient	Stations	Slopes (<i>b</i>)	
		Spring	Fall
Level 1	1	-0.13*	-0.60
	2	-0.30*	-0.80
Level 2	3	-0.03*	-0.24*
	4	-0.50*	0.11*
Level 3	5	-0.60	-0.61
	6	-0.77	-1.02
Level 4	7	-1.52*	n.d
	8	n.d.	n.d.

Discussion

The results of this study demonstrate that the salinity gradient determines patterns of variation both in the structure of benthic macroinvertebrates guilds and in their organization mechanisms. Species diversity and guild species richness decreased with increasing

salinity, in agreement with the literature (Jorcin 1999, Therriault, 2002). The statistical parameters of the size structure varied with salinity. These variations were more evident in spring. Variations in size structure width along a perturbation gradient have been shown in river ecosystems (Pinna and Basset, 1997).

Table 5. Significance values (*P*) of fitting tests for five models of niche apportionment proposed by Tokeshi (1999). Models are calculated for each season and each gradient level. DD: Dominance Decay; MF: MacArthur Fraction; RF: Random Fraction; RA: Random Assortment; DP: Dominance Pre-emption. *P* values greater than 0.05 correspond to models that cannot be rejected. (**P*<0.05; ***P*<0.01; ****P*<0.001; #-for this level no model could be applied).

	Spring				
	DD	MF	RF	RA	DP
Level 1	***	***	*	0.125	***
Level 2	***	***	**	0.466	***
Level 3	***	***	***	0.073	***
Level 4	***	***	***	***	0.340

	Fall				
	DD	MF	RF	RA	DP
Level1	***	***	***	***	***
Level 2	***	***	0.117	0.269	***
Level 3	***	***	***	***	***
Level 4 #	-	-	-	-	-

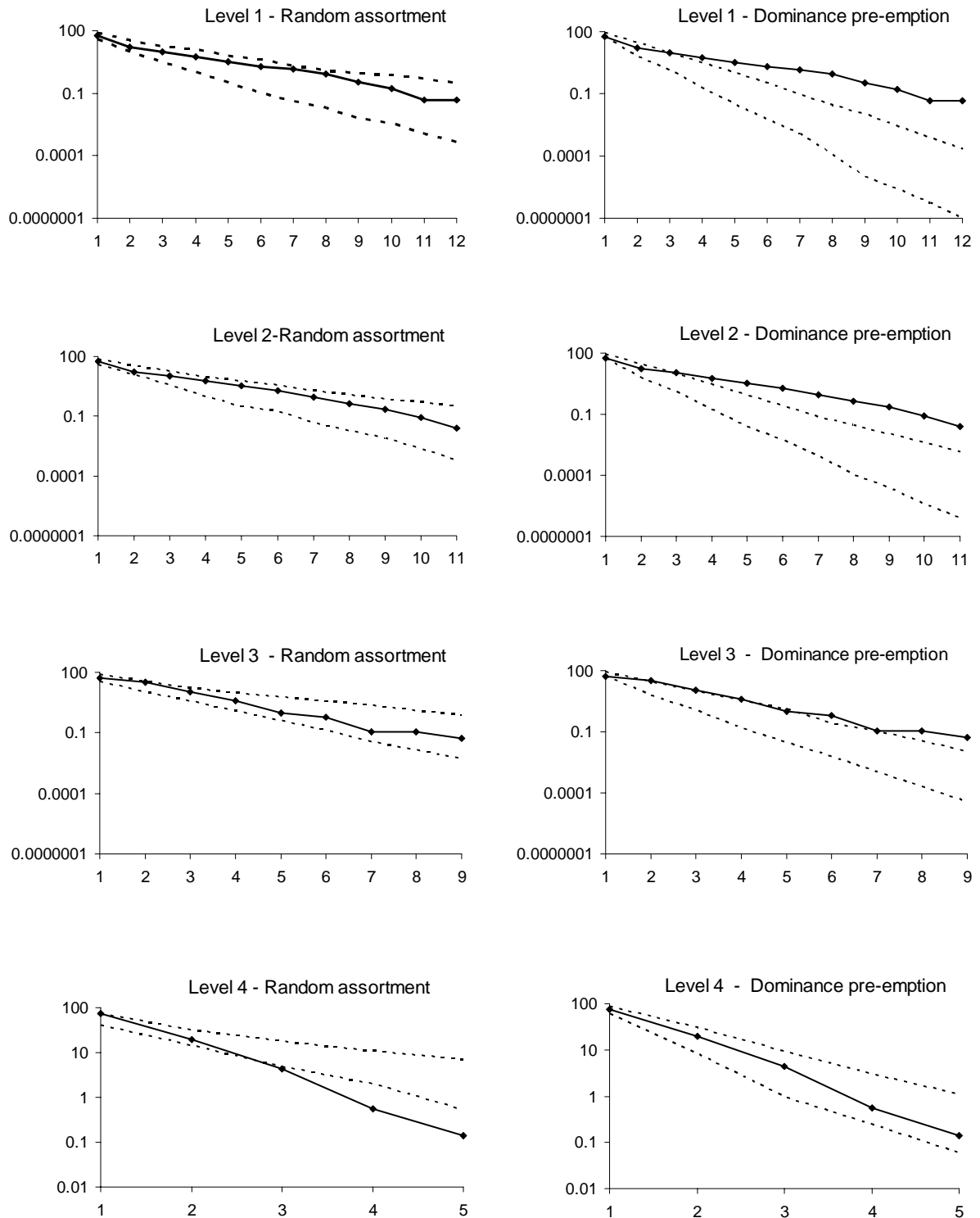


Figure 3. Graphic representation of fits resulting from two niche apportionment models (RA and DP) applied to each gradient level in the spring. Solid lines: observed result; dotted lines: confidence interval.

The body-size approach has been used in a number of more recent papers. It has been applied in a variety of ecosystem including an

oxygen minimum zone (Quiroga et al, 2005) and a deltaic environment (Akoumianaki et al, 2006). In this study, the body size oriented

mechanisms of benthic macroinvertebrates guilds, calculated as the difference between the slopes of the size-abundance relationships and their expected value of -0.75 , varied significantly along the salinity gradient in spring. The observed slope values were more negative at higher salinity values. This pattern is in agreement with the available literature data relative to impacted aquatic ecosystems characterised by relatively high occurrence of the smallest animals (Rasmussen, 1993).

The niche-oriented approach with the niche-apportionment model has been applied in other aquatic systems like ponds and lake for the study of dragonfly larvae community (Johansson et al, 2006), in river for Chironomidae (Tokeshi, 1990; Fesl, 2002). In this work for the first time these models have been used in a transitional water ecosystems.

Analysis of niche oriented organization mechanisms showed that the most frequently accepted model at the four gradient levels was Random Assortment. This model suggests a situation where there is little or no relationship between niche partitioning and relative species abundance. This situation may occur when the communities undergo stochastic variations on brief time scales of environmental forcing factors. Literature data suggest that the Random Assortment model provides a good explanation of the guild organization mechanisms of Chironomidae (Tokeshi, 1990; Schmid, 1997; Fesl, 2002). At the fourth gradient level, Dominance Pre-emption was the only model that could be accepted. This model describes as in the present case a situation where new species invade niche spaces previously utilized

by other species. In fall, Random Assortment was the only model that was accepted, but only at the second level. Comparative analysis of the body size oriented and niche oriented mechanisms suggested that, at lower gradient levels (low disturbance), guild organization was determined by size-based coexistence mechanisms (with the dominance of largest species), and was independent of any niche division. At higher gradient levels (high disturbance), guild organization was dependent on the dominance of the more resistant species (*Chironomus salinarius*). At intermediate gradient levels, both the body size oriented and niche oriented mechanisms seemed to indicate that interactions between species had little influence on guild organization.

Conclusions

In conclusion, this study suggests that in the Margherita di Savoia salt pans, organization mechanisms vary in relation to the salinity gradient. Hypersaline ecosystems seem to be suitable environments in which the responses of benthic macroinvertebrate guilds to increasing pressure, in both structural and dimensional terms, could be evaluated.

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