

RESEARCH ARTICLE

Habitat types and distribution of benthic macroinvertebrates in a transitional water ecosystem: Alimini Grande (Puglia, Italy)

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Abstract

- 1 - It is well-known that the variation patterns of structures and processes depend on scale – whether this be intrinsic or imposed by the observation methods. Transitional water ecosystems, being ecotonal systems, represent a suitable model for studying such relations.
- 2 - The aims of this study were: 1) to describe the macro-zoobenthic community of Lake “Alimini Grande” and 2) to analyze its patterns of spatial and temporal variation.
- 3 - The macro-zoobenthic community was sampled in three benthic habitat types (2 stations per type, five replicates per station) using a sediment box-corer, in two seasons (fall and spring).
- 4 - The density data were used for a qualitative-quantitative description of the community and subsequently underwent univariate and multivariate analyses to determine patterns of variation among replicates, stations, habitat types and seasons. A total of 28 taxa were recorded, subdivided into 3 feeding groups; filterers were the most common in terms of abundance and number of taxa.
- 5 - The distribution of benthic macroinvertebrates was found to be influenced by both temporal and spatial factors. The habitat type with the highest density and taxonomic richness exhibited significant intra-type heterogeneity of the community, suggesting that habitat type could be distinguished at a finer scale than the one previously adopted.

Keywords: habitat type, spatial distribution, benthic macroinvertebrates, transitional waters.

Introduction

To develop theories able to explain community organization and dynamics, it is necessary to detect and describe recurring patterns and to understand the underlying processes. In the last few decades the influence of the observation scale on the description of patterns has been widely recognized; indeed, description of environmental variability and predictability must encompass a suitable range of scales, which are relevant to the processes under investigation (Levin, 1992; Benedetti-Cecchi et al, 2005). At large spatial scales, species distribution is determined by geophysical and climatic forcing factors, and is closely related to

the dispersive abilities of the organisms (Pianka, 1966; Ricklefs, 1972, 1975). On the other hand, at small scales the processes involved are much more heterogeneous and are linked to the trophic, spatial and behavioural requirements of the organisms, as well as to stochastic processes (Tilman and Kareiva, 1997). The spatial and temporal heterogeneity of habitats and their related biotic and abiotic fluctuations seem to be the factors which, together with competitive and prey-predator interactions, locally determine the structure and organization of communities (Thrush, 1999). Transitional water ecosystems represent a particularly suitable model for studying the relationships between scale of observation and

patterns of organism distribution. They are ecotonal systems characterized by high structural heterogeneity and significant fluctuations in the chemical-physical parameters of water and sediments, even at small spatial and temporal scales (Basset et al, 2001). Such contexts enable an effective study of processes which, operating at small scales, may influence and counteract the general patterns determined by forcing factors acting at a higher scale. An important approach to selecting the spatial scales of observation is to identify environmental discontinuities and different habitat types. Attempts to devise a typological classification scheme for transitional water ecosystems have looked at inter-habitat variability, as in the "System of Venice" (Battaglia, 1959) in which the salinity range is used as a discriminating factor. They have also considered intra-habitat variability, as in "Confinement Theory" (Guelorget and Perthuisot, 1983) and in "Ergoline Theory" (Legendre and Demers, 1985), which classify ecosystems according to the rarefaction level of marine organisms or the existence of energetic gradients. In the context of marine-coastal ecosystem protection and conservation, a hierarchical habitat classification system has recently been proposed which is based on the identification of representative habitat types and on the assumption that benthic communities are strongly related to such types. (Roff and Taylor, 2000; Roff et al, 2003). The aim of the present paper was to describe the macro-zoobenthic community in a transitional water ecosystem of the Salento peninsula (Lake "Alimini Grande": Puglia, Italy) and to evaluate patterns of spatial and temporal variation within the system. As potential sources of spatial variability of benthic invertebrates we considered both confinement (as a function of distance from the lake's seaward inlet) and the heterogeneity of bottom substrates. The distribution of benthic invertebrates is known to be influenced by sediment grain size (Teske and Wooldridge, 2003) and by the presence of vegetation (Kafanov and Plekhov, 2001). Therefore, a habitat classification scheme was drawn up in accordance with Roff and Taylor (2000) and

Roff et al (2003), following a two-level factorial approach based on sediment grain size, and the presence and composition of benthic vegetation.

Materials and Methods

Study Area

Lake Alimini Grande is a transitional water ecosystem on the Adriatic coast of southern Italy (40°11'26.52" to 40°13'12.72"N, 18°26'13.64" to 18°27'19.88"E; Fig. 1). It has an irregular shape, a maximum depth of 3.5m (average 1.5m) and a surface area of 1.26km² which depends on both water level, which varies according to tidal cycles, and water inflow from tributaries (Sangiorgio et al, 2004). The lake receives freshwater inputs from a higher lake named 'Fontanelle' on the south western side and several drainage channels from the catchment basin on the northern side. The lake is separated from the sea by a narrow belt of sand dunes and is almost entirely surrounded by rocky shores. It is connected to the Adriatic sea by a 10m wide channel. Vegetation is not evenly distributed along the shore and consists of Mediterranean maquis (chaparral), mainly dominated by *Quercus coccifera* L., *Cistus monspeliensis* L., *Pistacia lentiscus* L., *Rosmarinus officinalis* L., *Erica arborea* L. and *E. verticillata* Forsskal. At the interface between the land and the aquatic ecosystem the presence of reed *Phragmites australis* (Cav.) Trin. ex Steud is relevant.

Experimental design

The experimental design considered variability between habitat types, variability at the intra-habitat scale deriving from differences between sampling stations located inside the same type, and intra-station variability among different replicates. Measurements were taken in two seasons. Habitat-type classification was based on the degree of confinement (expressed as the distance from the inlet connecting the lake to the sea) and the characteristics of the bottom substrate.

The latter criterion was evaluated using a two-level factorial approach (encompassing sediment grain size and presence of vegetation),

conceptually analogous to the one proposed by Roff and Taylor (2000) for marine-coastal ecosystems. In the present study, the structural zoobenthos component originally used by these authors was replaced with a structural vegetation component, held to be more

appropriate for transitional water ecosystems. Specifically, we defined habitat types using 3 levels of sediment grain size (mud, sand, rock) and 4 levels of vegetation (absent, macro algae, submerged macrophytes, emergent macrophytes).

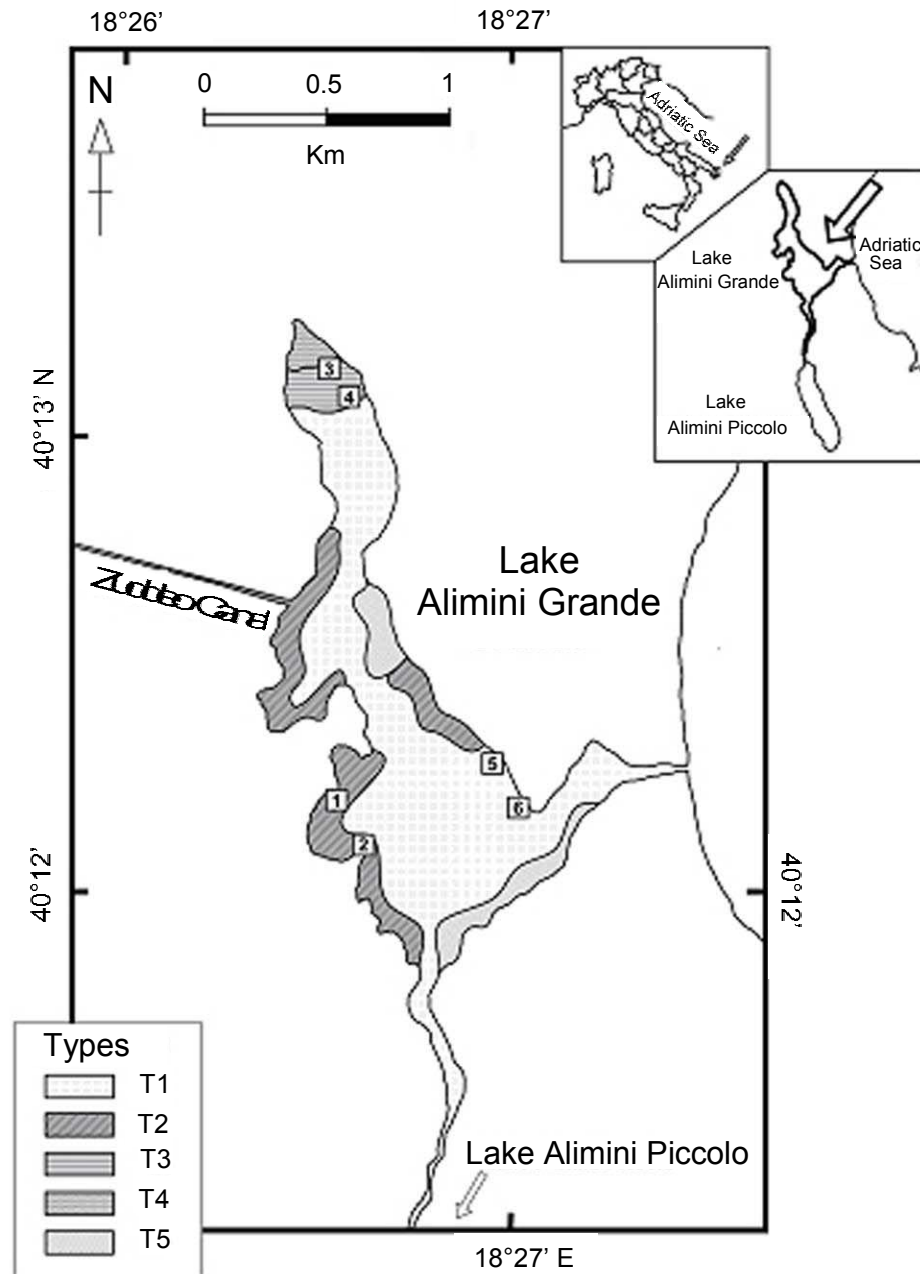


Figure 1. Map of study site with classification of habitat types and sampling stations (1, 2, 3, 4, 5, 6). T1=sand without vegetation; T2=sand with emergent macrophytes; T3=mud without vegetation; T4=mud with emergent macrophytes; T5=rock without vegetation.

By analysing sediment grain size (Tab. 1) and

visually determining the presence of vegetation

coverage associated with the sediment during a preliminary survey, the ecosystem was subdivided into five habitat types (Fig. 1): Type 1 – sand without vegetation; Type 2 – sand with emergent macrophytes; Type 3 – mud without

vegetation; Type 4 – mud with emergent macrophytes; Type 5 – rock without vegetation. In this paper we considered only the habitat types thought to be representative of the whole ecosystem: Types 1, 2 and 3.

Table 1. Abiotic parameters in water and sediment granulometry.

	T (°C)	Sal (PSU)	O ₂ (mg/L)	Clay (%)	Silt (%)	Sand (%)
Fall Type 1	14.31	31.45	8.65	2.0	14.2	83.8
Type 2	14.34	31.27	8.72	3.0	4.5	92.5
Type 3	15.42	28.27	8.52	12.8	51.5	35.7
Spring Type 1	17.69	28.41	7.10	2.0	14.2	83.8
Type 2	18.14	28.17	7.07	3.0	4.5	92.5
Type 3	17.87	24.40	7.26	12.8	51.5	35.7

The null hypothesis was that the structure of the macro-zoobenthic community did not show significant differences among habitat types and therefore the variability within each type was comparable to variability between types. To test this hypothesis we adopted a hierarchical sampling design considering type and station as factors. We sampled at 6 stations, 2 for each type, replicated 5 times in two seasons (November 2004 and April 2005); a total of 60 samples were collected.

The stations were situated at increasing distances from the seaward inlet of “Alimini Grande” (Fig. 1); the nearest stations were 5 and 6, belonging to type 1 (0.5km); stations 1 and 2, belonging to type 2, were at an intermediate distance (1.5km), and stations 3 and 4, belonging to type 3, were the most distant (2.2km).

Sampling methods and analyses

The macro-zoobenthic community was sampled using a standard hand-operated box-corer (sampled surface: 17x17cm). The samples were collected by penetrating 15cm into the sediment and were conserved in plastic bottles. To minimize disturbance to organisms and therefore the risk of sampling errors, operations were conducted from a rowing boat. For each station we measured temperature (°C), salinity

(PSU) and dissolved oxygen (mg/l) with a multiparametric probe (Tab. 1).

The samples were taken to the laboratory, washed with tap water on a 0.5mm sieve and fixed with 4% buffered Formaldehyde solution. Subsequently, organisms were sorted and selected from the sediment matrix under stereomicroscopes, identified by dichotomous keys and counted. Following Cummins and Klug (1979), an attempt was made to classify species into feeding groups: filterers, scrapers, shredders and predators. Filterers derive food material from the water or from the sediment, and the filter may consist of either parts of the body or manufactured devices such as silk nets; scrapers feed on the biofilm covering the surface of submerged structures such as stones and plant material; shredders chew CPOM (Coarse Particulate Organic Matter) as a food source and turn it into Fine Particulate Organic Matter or FPOM; and predators attack and engulf other macroinvertebrates and occasionally fish as a main food source. Yet, for statistical analyses, scrapers and shredders were grouped, since for many sampled species unambiguous information on their trophism was not available. Furthermore, it is well known that the approach based on trophic-functional groups - originally developed for freshwaters - is subjected to criticism and currently revised;

indeed it has been recognized that the benthic macroinvertebrates are characterized by high trophic plasticity at different levels of taxonomic organization (Dangles, 2002 and MacNeil et al, 1997 for gammarideans).

Data Analysis

Macro-benthic community structure was analyzed by calculating, for each sampling unit, density (ind/m²), number of taxa (S), Shannon-Wiener species diversity (H') and Pielou evenness (J). The relative contribution of feeding groups was evaluated in terms of density and number of taxa. Data were analyzed with two way nested ANOVA using Station Factor (two levels: random) nested within Type Factor (three levels: fixed). Differences between seasons were tested by one-way ANOVA. The heteroschedasticity of data was tested with the Cochran C test. Significance detected by ANOVA was analyzed thoroughly by post-hoc tests (Tukey HSD-Honest Significance Difference). The species responsible for

dissimilarities among types were identified using the similarity percentages procedure SIMPER (Clarke, 1993). The Bray–Curtis similarity index was used to investigate similarities among samples in each station separately.

Sample size valuation

In the determination of the overall heterogeneity of macroinvertebrate community, the congruity of the sample size was evaluated by constructing and analyzing rarefaction curves (Fig. 2). The number of collected samples, determined by the experimental design, proved to be exhaustive for the evaluation of species richness; indeed, all the rarefaction curves for the three habitat types reached a plateau (Fig. 2). The curves for Types 1 and 3 reached saturation after a few replicates, while for Type 2 the number of replicates sampled seemed to be merely adequate to represent species richness.

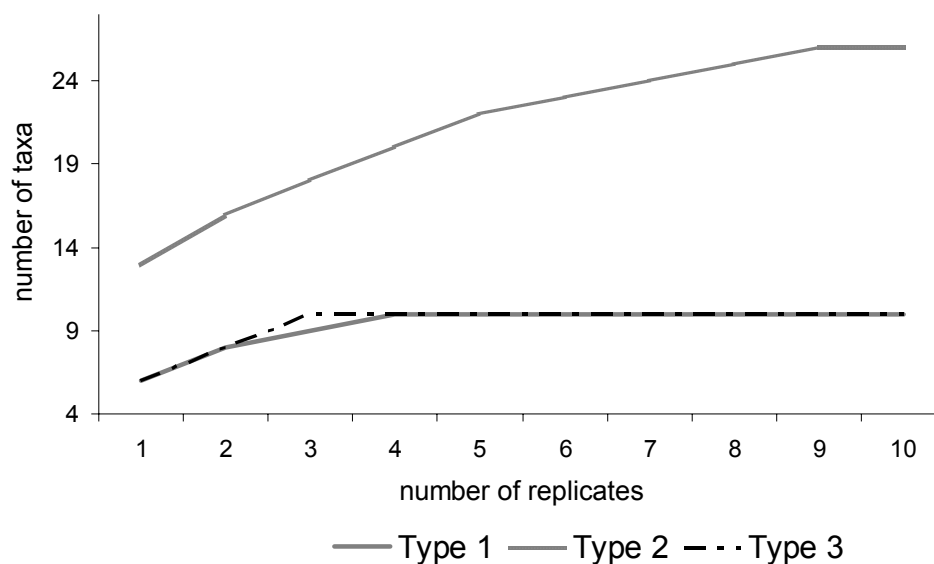


Figure 2. Rarefaction curves calculated for each habitat type.

Results

In the 60 samples collected in fall 2004 and spring 2005, a total of 4181 individuals were found, belonging to 28 taxa; of these 17 were classified at the species level, 5 at the genus level and the remaining were assigned to higher

taxonomic groups (from Sub-Family to Phylum) (Tab. 2). The identified taxa belonged to the following Phyla: Arthropoda, Annelida, Mollusca and Sipuncula. Half the observed taxa belong to the Class Bivalvia and Malacostraca.

Table 2. Taxonomic list for all sampling stations. Density and frequency of all taxa are shown for both sampling periods (F=filterers, S/S=scrapers/shredders, P=predators).

Taxa	GF	Fall		Spring	
		density (ind/m ²)	frequency (%)	density (ind/m ²)	frequency (%)
<i>Loripes lacteus</i>	F	402.54	96.67	441.75	86.67
Haplotaxida	F	91.12	43.33	1011.53	100.00
<i>Ficopomatus enigmaticus</i>	F	21.91	16.67	1.15	3.33
<i>Tapes sp.</i>	F	8.07	16.67	-	-
<i>Corophium sp.</i>	F	2.31	6.67	2427.91	33.33
Sipuncula	F	1.15	3.33	-	-
<i>Abra segmentum</i>	F	-	-	40.37	56.67
<i>Cerastoderma glaucum</i>	F	-	-	11.53	23.33
<i>Dosinia lupinus</i>	F	-	-	5.77	16.67
Enchytraeidae	F	-	-	8.07	3.33
<i>Gastrana fragilis</i>	F	-	-	2.31	6.67
<i>Mytilaster sp.</i>	F	-	-	5.77	10.00
<i>Glycera sp.</i>	P	28.84	40.00	9.23	23.33
<i>Nereis diversicolor</i>	P	6.92	16.67	12.69	20.00
<i>Austropotamobius pallipes</i>	P	1.15	3.33	-	-
Tanypodinae	P	-	-	1.15	3.33
<i>Sphaeroma hookeri</i>	S/S	2.31	6.67	-	-
<i>Nassarius sp.</i>	S/S	-	-	31.14	36.67
<i>Cyclope neritea</i>	S/S	-	-	2.31	6.67
Chironomidae	S/S	-	-	1.15	3.33
<i>Chironomus plumosus</i>	S/S	-	-	68.05	20.00
Diamesinae	S/S	-	-	4.61	6.67
<i>Bittium reticulatum</i>	S/S	-	-	43.83	30.00
<i>Gammarus insensibilis</i>	S/S	-	-	3.46	3.33
<i>Hydrobia ventrosa</i>	S/S	-	-	9.23	6.67
<i>Limnoria lignorum</i>	S/S	-	-	8.07	3.33
<i>Microdeutopus gryllotalpa</i>	S/S	-	-	42.68	10.00
<i>Tanais dulongii</i>	S/S	-	-	62.28	26.67

Classes found in all three habitat types at both sampling times were Bivalvia and Oligochaeta. Gastropoda and Insecta were found only during spring and not in every type; however, where they were found, they were represented by a number of taxa comparable to that of the most abundant and frequent classes. Malacostraca were recorded only in type 1, with a higher number of taxa in spring than in fall. There were 12 taxa of filterers, 12 taxa of scrapers/shredders, and 4 taxa of predators (Tab. 2).

Density, number of taxa, diversity (Shannon-Wiener index) and evenness (Pielou index) were

used as descriptors of community structure. Data underwent Analysis of Variance to discriminate between habitat types, sampling stations and replicates. After testing the occurrence of statistically significant differences between sampling seasons (one way ANOVA, P<0.001), two way nested ANOVA (type and station) was performed for each season. All descriptors differed significantly among habitat types and stations (Tab. 3; Fig. 3).

The “type” factor was significant in both seasons. The “station” factor nested in “typology” was also strongly affected by

season, being significant in fall in all cases, but almost never significant in spring.

Density of collected macrozoobenthos ranged from 69.20 ind/m² to 1695.50 ind/m² in fall and from 726.64 ind/m² to 34013.84 ind/m² in spring. Density was significantly different among types (Tab. 3) both in spring and fall. Post-hoc tests revealed that, in fall, density in Type 1 (280.28 ind/m²) was significantly lower than other types, and that, in spring, density in Type 2 (9380.62 ind/m²) was considerably higher than density in either Type 1 (1806.23 ind/m²) or Type 3 (1581.31 ind/m²). In spring,

Type 2 showed significant differences between sampling stations (Tab. 3) (Fig. 3) due to an extremely clumped distribution of *Corophium sp.*, which reached density values of 14186.85 ind/m² in station 1 and just 380.62 ind/m² in station 2. Analyzing spatial variability at a finer scale, it was observed that in station 1 the extremely high density of *Corophium sp.* did not occur in every replicate, but only in three of them; the other two replicates in station 1 showed density values not much higher than the replicates in station 2.

Table 3. Significance of nested ANOVA (factors: station nested in type) applied to community macrobenthos descriptors (*P<0.05; **P<0.01;***P<0.001)

Variable	Level	Fall		Spring	
		df	F	df	F
density (n.ind./m ²)	types	2	5.29*	2	6.76**
	stations	3	0.17 n.s.	3	7.17**
n.taxa	types	2	15.16***	2	33.78***
	stations	3	5.71**	3	2.11 n.s.
d	types	2	17.40***	2	22.93***
	stations	3	8.51***	3	0.13 n.s.
J'	types	2	32.48***	2	5.63**
	stations	3	21.31***	3	2.79 n.s.
H'	types	2	28.07***	2	6.61**
	stations	3	14.31***	3	1.28 n.s.
density filterers	types	2	4.64*	2	6.20**
	stations	3	0.18 n.s.	3	6.85*
density s/s	types	2	-	2	19.26***
	stations	3	-	3	12.95***
density predators	types	2	7.75**	2	12.07***
	stations	3	1.02 n.s.	3	2.34 n.s.

Assigning individuals to their feeding group, we observed that the mean density of filterers was always greater than that of scrapers/shredders and predators (Tab. 3). In both sampling seasons, the number of taxa in Type 2 was significantly higher than in the others (Tukey post-hoc test, P<0.01). Differences among habitat types were greater in spring, when the number of taxa per type varied from 7 to 22, than in fall, when we observed values ranging between 5 and 9. The significantly higher number of taxa in Type 2 during spring was due mainly to an increase in the number of

scraper/shredder taxa, which increased from only 1 in fall to 12 in spring. The analysis of variance applied to the indexes of diversity and evenness in fall showed significant differences among both types and stations (Tab. 3); post-hoc tests showed that each type was different from the others, with the H' and J' values of Type 3 significantly lower than other two (Fig. 3). Moreover, within Type 1 the stations differed significantly, in terms of both H' and J' (Fig. 3). In spring, H' in Type 1 was different from the other two. Analysis of the contribution of each taxon to dissimilarity among types

showed that differences in fall between Types 2 and 3 and between Types 2 and 1 were due to the species *Loripes lacteus* and the order

Haplotaxida, whereas the differences between Types 3 and 1 were attributable to the species *Loripes lacteus* and *Ficopomatus enigmaticus*.

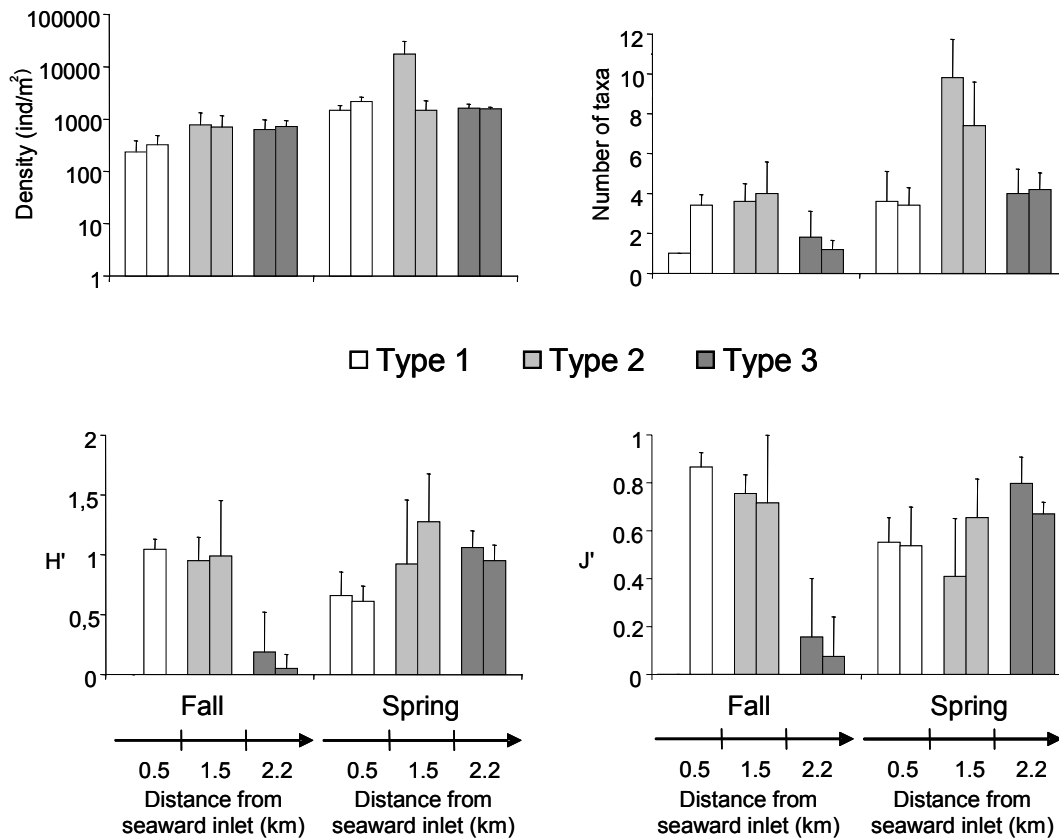


Figure 3. Community structure attributes: density, number of taxa, Shannon–Wiener diversity index (H'), Pielou index (J') in relation to type, sampling season and distance of stations from seaward inlet of Lake Alimini Grande.

In spring, the dissimilarity between Types 2 and 3 were mainly due to the species *Loripes lacteus* and *Corophium sp.*; in contrast, the differences between Types 2 and 1 were primarily attributable to the species *Corophium sp.* and the order Haplotaxida, and the differences between Types 3 and 1 were due to the order Haplotaxida and the species *Loripes lacteus*. The complete results of the SIMPER procedure are reported in table 4. The values of the benthic macroinvertebrate community descriptors did not vary significantly along the confinement gradient (i.e. the distance of sampling stations from the lake's seaward inlet), with the exception of the diversity indexes in fall.

Discussion

The results of this study highlight two fundamental points: 1) the approach to the classification of transitional water ecosystems based on habitat types seems to explain the heterogeneity of the benthic macroinvertebrate community in Alimini Grande more effectively than traditional confinement theory; 2) among the identified habitat types in Alimini Grande, sand with emergent macrophytes has the highest density and species diversity. Regarding the first point, the results of the present study show that in Alimini Grande habitat type constitutes a scale of spatial variation in macroinvertebrate benthic community which is independent of the

classifications of transitional water ecosystems based on “Confinement Theory” (Guelorget and Perthuisot, 1983) and the “System of Venice” (Battaglia, 1959).

This is supported by the fact that in Habitat Type 3, the most confined and distant from the seaward inlet, density was no lower than the

other two Types in either spring or fall, and diversity and evenness were lower only in fall. In contrast with expectations from Confinement Theory, in spring, differences in terms of species richness and diversity between Types 1 and 3 were not significant (post-hoc test $P > 0.05$).

Table 4. SIMPER analysis. List of species showing highest contribution to average dissimilarity between types. $S_i\%$: contribution of i th species to total dissimilarity, also expressed as cumulative percentage ($\sum S_i\%$).

		Fall					Spring		
		Species	$S_i\%$	$\sum S_i\%$			Species	$S_i\%$	$\sum S_i\%$
Mean Dissimilarity between types 2 and 3 = 47.40		<i>Loripes lacteus</i>	48.74	48.74	Mean Dissimilarity between types 2 and 3 = 74.44		<i>Loripes lacteus</i>	36.22	36.22
		Haplotaxida	31.99	80.73			<i>Corophium sp.</i>	28.8	65.03
		<i>Glycera sp.</i>	12.1	92.83			Haplotaxida	16.82	81.85
Mean Dissimilarity between types 2 and 1 = 54.77		<i>Loripes lacteus</i>	34.97	34.97		<i>Bittium reticulatum</i>	4.58	86.43	
		Haplotaxida	26.95	61.92		<i>Abra segmentum</i>	2.95	89.38	
		<i>Ficopomatus enigmaticus</i>	16.43	78.35		<i>Tanais dulongii</i>	2.29	91.67	
		<i>Glycera sp.</i>	11.73	90.09		<i>Corophium sp.</i>	35.22	35.22	
Mean Dissimilarity between types 3 and 1 = 36.41		<i>Loripes lacteus</i>	48.71	48.71	Mean Dissimilarity between types 2 and 1 = 60.88		Haplotaxida	32.8	68.03
		<i>Ficopomatus enigmaticus</i>	24.51	73.22			<i>Loripes lacteus</i>	15.51	83.54
		<i>Glycera sp.</i>	9.41	82.62			<i>Tanais dulongii</i>	2.81	86.34
		Haplotaxida	8.37	90.99			<i>Chironomus plumosus</i>	2.14	88.48
Mean Dissimilarity between types 3 and 1 = 47.64						<i>Abra segmentum</i>	1.51	90	
						<i>Nassarius sp.</i>	1.45	91.44	
						Haplotaxida	47.07	47.07	
						<i>Loripes lacteus</i>	36.99	84.07	
						<i>Bittium reticulatum</i>	7.39	91.46	

In the studied system, salinity varied along a gradient broadly corresponding to that of confinement, with higher values in habitat types near the seaward inlet and lower values far away from it. Thus, spatial variations in the distribution of benthic macroinvertebrates did not seem to follow the typical pattern of salinity variations induced by tidal oscillation.

Therefore, the patterns of macrozoobenthic community distribution are not consistent with the variations expected from “Confinement Theory” and the "System of Venice."

Regarding the second point of discussion, the results of the present study showed that Type 2 (sand with emergent macrophytes) was the habitat with the highest density and diversity values. There are several factors that may determine the observed variations among the

examined habitat types. The presence of macrophytes, interfering with the hydrodynamic regime, considerably changes the water column transparency and the deposition and accumulation on the lake bed of organic matter, which constitutes a major trophic resource for many benthic macroinvertebrates.

The tissues produced by plants in the growth phase constitute a direct trophic resource for scrapers and shredders and, as a result of plant senescence, enrich the detrital component. Furthermore the presence of a complex system of roots, stems and leaves promotes habitat structuring and stability, enabling more species to meet the combination of environmental conditions necessary for the settlement, recruitment and the maintenance of their populations. Such habitat structuring provides

shelter from predation and promotes niche partitioning, attenuating inter-species competition processes (Diehl, 1992).

Our results suggest that analysis of habitats within transitional water ecosystems, classified on the basis of substrate, represents an innovative approach to define the most suitable methods and experimental design for the description of benthic macroinvertebrate communities at more detailed spatial scales.

In the case of Alimini Grande, measures to restore and safeguard the ecosystem must seek to maintain the heterogeneity of aquatic habitats, particularly those habitats exposed to perturbations (e.g. destruction of reed beds). In addition, measures need to be adopted which facilitate the enlargement of habitats characterized by the highest diversity and stability.

In this case study, the spatial scale best suited to describe benthic macroinvertebrate variability seems to be substrate type rather than the gradients of salinity and confinement.

The higher heterogeneity observed in fall within every habitat type (i.e. between stations) seems consistent with the sources of variation mentioned. Indeed, it seems probable that plant

senescence during such season, which makes habitats less heterogeneous, may involve other factors, operating on different spatial scales, which influence the distribution of benthic fauna.

Conclusions

In conclusion, this study makes a contribution to the definition of criteria for choosing the correct scale of observation to adopt in monitoring and maintenance programs concerning biodiversity in transitional water ecosystems.

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